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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 15 FEBRUARY 1957 No. 2

NUTRITIONAL ROLE OF FOLIC ACID

The hematopoietic efficacy of folic (pteroylglutamic) acid in various types of megaloblastic anemia has long since been firmly established; in fact, the megaloblastic anemias of pregnancy and of infancy almost always reflect a primary deficiency of this vitamin. Even in pernicious anemia, in which the primary need clearly is for vitamin B_{12}, folic acid almost invariably will induce, and usually will maintain for months or even years, adequate erythropoiesis. It is well known, of course, that folic acid, unlike vitamin B_{12}, is quite unable to arrest the neuropathologic manifestations not infrequently encountered in this disease. In fact, in patients initially demonstrating only hematologic signs of pernicious anemia, the administration of folic acid (without vitamin B_{12}) may be followed by the appearance of the manifestations of combined system disease. Before this situation is discussed, thereby giving the hematopoietic functions of folic acid perhaps undue emphasis, it may be well to review briefly the biochemical functions of this vitally important compound.

Folic acid, like other members of the vitamin B-complex, serves as a precursor of an essential cofactor or group of related cofactors, without which a variety of reactions cannot proceed at a rate compatible with life. These reactions are concerned with the coenzymic transfer of certain "one-carbon units" involved in the biosynthesis of a variety of compounds upon which the reproduction of cells is dependent (A. D. Welch and C. A. Nichol, *Ann. Rev. Biochem.* **21,** *633 (1952)*); the formimino group (—CH=NH) also is transferred by means of a conezyme derived from folic acid (J. C. Rabinowitz and W. E. Pricer, Jr., *J. Am. Chem. Soc.* **78,** *4176, 5702, 5705 (1956)*). Although the exact structure of these folic acid-derived co-factors has not been completely established, many of the reactions in which they participate have been studied rather extensively; in mammals these include reactions involved (a) in the biosynthesis of various purine-containing compounds (found in all nucleic acids and in a number of essential cofactors), (b) in the formation of a substance uniquely found in deoxyribonucleic acid, i.e., thymidine, (c) in the interconversion of glycine and serine, (d) in the catabolism of certain amino acids, and (e) in the generation of the labile methyl group. Moreover, the list is probably not complete, for it is not yet possible in animals to nullify the violently toxic effects of a potent antagonist of folic acid such as Amethopterin ('Methotrexate') by supplying a mixture of those compounds known to depend upon folic acid-derived coenzymes for their biosynthesis. Although it cannot be stated unequivocally that there are one or more unknown essential compounds of this class, for various complicating factors affecting such a conclusion must be considered, the important point to establish here is that derivatives of folic acid are absolutely essential for various reactions of normal life. Consequently, conclusions concerning the need for and the appropriateness of supplying this vitamin must rest on this essentiality, rather than on hematologic considerations and the complicated problems of pernicious anemia.

Concerning the suggested participation of vitamin B_{12} in the formation or function of a coenzyme derived from folic acid (A. D. Welch and R. W. Heinle, *Pharmacol. Reviews* **3,** *345 (1951)*), definitive evidence is still not available. Intriguing but circumstantial support for such a view is to be found in the results of a study (by D. H. Clement, A. D. Welch and C. A. Nichol) of

a case of severe juvenile pernicious anemia (for essential aspects *see* C. A. Nichol, Symposium on Vitamin Metabolism, National Vitamin Foundation, New York, *Nutrition Symposium Series* **13**, 77 (1956)). The patient, a four-year-old boy, subsequently shown not to utilize orally administered 0.5 μg. doses of vitamin B_{12}-Co^{60}, developed a very severe clinical and hematologic relapse during prolonged oral therapy with folic acid, ascorbic acid and vitamin B_{12}. Following a single intramuscular injection of vitamin B_{12} (30 μg.), while daily oral dosage with folic acid and ascorbic acid (15 mg. and 75 mg., respectively, in three divided doses) was continued, a very rapid and complete clinical and hematologic remission resulted. Prior to the parenteral administration of vitamin B_{12}, the urine contained relatively large amounts of a structurally unidentified, microbially active, heat-labile, reduced form of folic acid (disclosed chromatographically), but 48 hours following the injection of vitamin B_{12}, the urine contained only those metabolites of folic acid which occur normally. Conceivably, the severity of the deficiency of vitamin B_{12} was so great in this child that the capacity to convert folic acid to a functional coenzyme, or to maintain an adequate concentration of the latter, had been lost completely. This could have resulted in the urinary elimination of an otherwise transiently existing intermediary metabolite of folic acid. Thus, possible evidence of a dependence upon vitamin B_{12} for the availability in man of a functional derivative of folic acid may have been provided; earlier studies in swine had suggested a similar interdependence of these vitamins (R. W. Heinle, A. D. Welch and J. A. Pritchard, *J. Lab. Clin. Med.* **33,** *1647 (1948)*; R. W. Heinle, A. D. Welch and H. L. Shorr, *J. Lab. Clin. Med.* **34,** *1763 (1949)*). It is requested that those who encounter cases of pernicious anemia refractory to folic acid, particularly in children, communicate with the author of this article, in order that the observations described above may be confirmed and extended.

We may now return to the question of the "danger" to the nervous system of man which possibly may be imposed by the increasingly wide-spread use of folic acid. Is there a hazard, and, if so, how great is it?

There have been many reports in which the development or intensification of manifestations of combined system disease during therapy of pernicious anemia with folic acid have been described; among these have been those of R. W. Heinle and A. D. Welch, (*J. Am. Med. Assn.* **133,** *739 (1947)*); C. F. Vilter, R. W. Vilter, and T. D. Spies, (*J. Lab. Clin. Med.* **32,** *262 (1947)*); J. F. Ross, H. Belding, and B. L. Paegel, (*Blood* **3,** *68 (1948)*); M. C. G. Israëls and J. F. Wilkinson, (*Brit. Med. J.* **2,** *1072 (1949)*); S. O. Schwartz, S. R. Kaplan and B. E. Armstrong, (*J. Lab. Clin. Med.* **35,** *894 (1950)*); C. L. Conley and J. R. Krevans, (*New Engl. J. Med.* **245,** *529 (1951)* and (*Ann. Int. Med.* **43,** *758 (1955)*). On the other hand, many patients with pernicious anemia have been maintained hematologically for several months (or even years) by treatment with folic acid alone, without developing evidence of neuropathologic changes; thus, in a series of 41 patients given folic acid in doses of 10 to 40 mg. daily and observed for one year (R. W. Heinle, J. T. Dingle and A. S. Weisberger, *J. Lab. Clin. Med.* **32,** *970 (1947)*) neurological relapse occurred in only three; also, in the series of 98 patients observed by Schwartz *et al.* for up to 3½ years on a daily dosage regimen of 5 mg. of folic acid, and among which 32 relapsed neurologically, 12 patients were maintained satisfactorily for the entire period, both hematologically and neurologically. Similarly, 4 of the 20 patients of Israëls and Wilkinson were quite well after 2 to 3 years on 5–20 mg. of folic acid daily.

Although it is clear that certain neurons of the central nervous system have a peculiar dependence upon vitamin B_{12}, or a product

of its catalytic activity, the expression of a deficiency by neuropathologic changes and the time required for their development are quite unpredictable when folic acid is given continuously without vitamin B_{12}. Presumably the differences encountered amongst patients are reflections of variations in the amounts of vitamin B_{12} absorbed by pernicious anemic individuals, with variable deficiencies in the secretion of intrinsic factor, and perhaps also of variations in unknown factors influencing the rate of loss of vitamin B_{12} from the tissues.

Only recently has evidence been adduced which suggests that the administration of folic acid actually may influence the metabolism of vitamin B_{12} in patients with pernicious anemia (A. A. Lear and W. B. Castle, *J. Lab. Clin. Med.*, **47**, *88 (1956)*); the findings suggested that the level of vitamin B_{12} in the serum of such patients may be reduced by the prolonged administration of folic acid. In this study, 35 patients who had been maintained in remission for two years or more with *monthly* injections of either vitamin B_{12} or liver injection, U.S.P., in amounts supplying 15 μg. of the vitamin, had an average concentration of 190 (range: 10 to 1050) $\mu\mu$g. of vitamin B_{12} per ml. of serum. After the parenteral administration for an additional period of six months of either vitamin B_{12} or liver extract, *twice monthly*, in doses supplying 60 μg. of vitamin B_{12} per month, the average concentration in 31 of these individuals had increased to 466 (range: 78 to 1840) $\mu\mu$g. per ml. of serum; the individual values rose in all cases. During an immediately subsequent *second* period of six months, the same dosage of vitamin B_{12} was continued, but, *in addition*, 5 mg. of folic acid was given daily by mouth. At the end of this second period the average serum concentration of 29 of the patients had *dropped* from 466 to 276 (range: 116 to 745) $\mu\mu$g. of vitamin B_{12} per ml. Even if only those 27 individuals who contributed to each of the three determinations are considered, the average values were 202, 414 and 280 $\mu\mu$g., respectively. Among these 27 cases, 9 actually demonstrated, at the end of the second period, a relatively small *rise* in vitamin B_{12} values (average, 59) (range: 1 to 212) $\mu\mu$g.), but, in the majority (18 patients) the average reduction in the vitamin B_{12} concentration in the serum was to one-half that obtained at the end of the first six-month period, i.e., from 578 (range: 221 to 1360) $\mu\mu$g. to 291 (range: 116 to 745) $\mu\mu$g. per ml.

Quite legitimately, it may be asked what would have happened to the levels of vitamin B_{12} in the serum during the second six-month period if folic acid had *not* been given in addition to vitamin B_{12}? Although the data of Lear and Castle afford an element of probability that the administration of folic acid reduced the level of vitamin B_{12} in the serum, this has not been established. If this were proved to be the case, and regardless of the mechanism involved, one could speculate concerning the effect of dosage with folic acid on the amount of vitamin B_{12} available to neural tissues in patients with pernicious anemia given folic acid *without* vitamin B_{12}. Obviously, further work is needed, for the data provided by Lear and Castle are provocative.

It is not legitimate, however, to take the position that folic acid is a dangerous substance from which human beings *in general* should be protected, despite the convincing evidence that treatment with folic acid alone enhances the possibility of the development of combined system disease in patients with pernicious anemia. Certainly, in those individuals given adequate amounts of vitamin B_{12} there appears to be no evidence of hazard from the additional use of folic acid. Thus, the problem as it relates to the *very* small percentage of human beings who have (or will develop) the disease now inappropriately termed pernicious anemia, is one primarily of diagnosis in the *absence* of the typical dyscrasias of the blood and bone marrow. Obviously, in the presence of mani-

festations suggestive of combined system disease, adequate parenteral therapy with vitamin B_{12} is indicated, whether or not other indications of pernicious anemia are present. For those having access to the appropriate facilities, a determination of the level of vitamin B_{12} in the serum or a test of the ability to absorb orally administered vitamin B_{12}-Co^{60} (R. W. Heinle, A. D. Welch, V. Scharf, G. C. Meacham and W. H. Prusoff, *Tr. Assn. Am. Physicians*, **65,** *214 (1952)*; R. F. Schilling, *J. Lab. Clin. Med.*, **42,** *860 (1953)*) makes a reliable diagnosis of pernicious anemia an attainable goal, even in the complete absence of the characteristic hematologic manifestations of this disease.

It is now quite clear that only a very small percentage of the population has a deficiency of intrinsic factor, and, therefore, an abnormally low capacity for the absorption of ingested vitamin B_{12}. Accordingly, it appears unjustified, in order to protect this very small group of individuals, to exclude another essential vitamin, folic acid, from all multivitamin preparations (as has been proposed), even though these preparations supply many materials of little or no value for the great majority of our population. Certainly, it is no more logical to exclude folic acid from a truly multivitamin preparation than it is to add to it, *for general use*, an expensive extract containing intrinsic factor, in order to insure the absorption of vitamin B_{12} by a very small group of defective individuals. A well balanced diet appears but rarely to require supplementation with *any* member of the vitamin B-complex and the supposed "evils" of excess folic acid probably could best be avoided by a dependence upon a good diet rather than upon capsules as a source of essential vitamins.

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FAT ABSORPTION IN SEVERE MALNUTRITION

During the refeeding program for children with severe malnutrition the regimen commonly employed is low in fat, since there is a clinical impression that children suffering from malnutrition develop steatorrhea when fed moderate amounts of fat. This belief is based on the fact that, since these children already have increased amounts of fat in the feces, feeding a diet low in fat would reduce the fecal fat level towards normal. With lower fat levels in the feces it was hoped that a better handling of other foodstuffs would be achieved.

A. W. Chung and collaborators (*Pediatrics* **7,** *491 (1951)*) studied fat tolerance in children with steatorrhea, 3 individuals with idiopathic celiac disease and 3 with cystic fibrosis, who were maintained on fat intakes between 25 and 165 g. per day. They noted that when the fat content of the diet was increased there was a greater amount of fat absorbed. These diets were well tolerated by the patients. It was concluded that the hypothesis that dietary fat leads to fat intolerance was found wanting under these conditions.

The work of F. Gomez, R. R. Galván, J. Cravioto, and S. Frenk (*Pediatrics* **13,** *548 (1954)*) offers some explanation of why there is an increased fat content in the feces of severely malnourished children. They found that the activity of amylase, trypsin and lipase was markedly reduced in the duodenal contents of 40 children with advanced severe malnutrition when compared with either the same group on recovery or normal individuals. The greatest decrease in enzyme activity was found in the lipase. There the activity fell to about 10 per cent of that noted in the normal individual.

Recently F. Gomez and associates (*Lancet* ***II,*** *121 (1956)*) reported the effects of feeding

increasing amounts of fat to chronically severely malnourished children. Fourteen Mexican children were studied, all of whom showed the main signs and symptoms of severe chronic malnutrition. Metabolic balance technics were used to study fat absorption. Feces were collected for periods of four days using carmine as a marker and were analyzed for fat. The amount of fat absorbed during the period was considered to be the difference between the amount fed and that found in the feces. All the children were studied at least twice, once on admission to the hospital and again when the child was in the recovery stage. Ten of the 14 children were also studied two weeks after they were admitted to the hospital for therapy.

The amount of fat in the initial diets varied from 5 to 42 g. per four-day period but in the majority of the cases remained below 20 g. About 40 per cent of the ingested fat was found in the feces of the malnourished individuals. In spite of losing nearly half of the fat intake in their feces, these children, with only a few exceptions, did not show visible steatorrhea, although by chemical analysis there were abnormal amounts of fat in the feces. In the period which began two weeks after admission the average absorption of fat for these individuals increased to 65 per cent of the ingested fat. At this time the patients' intake was usually greater than that received on admission. Finally, when the patients were in the recovery phase they were absorbing about 80 per cent of the fat in the diet, a figure which is approaching the normal value of about 95 per cent.

These changes can best be illustrated by using data from an individual case. On admission he was ingesting 29.4 g. of fat per four-day period and losing 14.8 g. in the feces (absorbing 48.3 per cent). Two weeks later he was taking in 50.2 g. of fat and losing 13.4 g. in the feces. During this period he was absorbing 73 per cent of the fat, in spite of having fecal losses of comparable amounts to those seen on admission. On recovery he was consuming 141.9 g. of fat and losing 14 g. in the feces, an absorption of 90 per cent.

Gomez and co-workers then plotted the fat intake against the fat absorption, expressing both in grams, and found that there was a direct linear correlation. That is, the amount of fat absorbed was related to the amount ingested. By increasing the fat intake, the amount absorbed also increased. This relation held for all three groups examined, the malnourished patients studied immediately on admission, those studied after two weeks of therapy, and the recovered patient. Thus while there apparently is a marked defect in the absorption of fat in severely malnourished individuals, increasing the fat intake of the diet will also increase the amount of fat absorbed.

The children who developed severe chronic malnutrition had been living on diets which were deficient in practically all nutrients including calories. Fat in addition to being one of the best sources of calories, on a weight basis, also meets the needs for certain noncaloric functions. The present use of low fat diets in such individuals perhaps needs further investigation to determine whether the best therapeutic and nutritional regimen is being employed in the treatment of chronic malnutrition.

NUTRITIONAL PERNICIOUS ANEMIA

It has been estimated that the normal diet contains 8 to 15 micrograms of vitamin B_{12} daily, an excess of that needed to prevent pernicious anemia. The relatively small daily requirements for this vitamin make it unlikely that any appreciable number of cases of pernicious anemia will result from insufficient exogenous vitamin B_{12}. However, low serum concentrations of vitamin B_{12} have been described in a group of vegetarians

(*Nutrition Reviews* **14,** *73 (1956)*), indicating the effect of the diet.

The development of the technics for measuring vitamin B_{12} absorption has led to the description of 2 patients with pernicious anemia of nutritional origin. M. Pollycove, L. Apt, and M. J. Colbert (*New Engl. J. Med.* **255,** *164 (1956)*) studied a patient of 60 who had eaten no animal or dairy products for ten years. He entered the hospital critically ill with stupor, hypotension, weakness and neurologic abnormalities. His hemoglobin was 2.7 g. per cent and his red cell count was 0.65 million per cubic millimeter. Examination of the blood film and bone marrow showed changes consistent with pernicious anemia. The serum vitamin B_{12} level was 70 micrograms per cent. Normal serum vitamin B_{12} concentration averages 530 micrograms per cent (*Nutrition Reviews* **13,** *100 (1955)*). The patient made a prompt response to parenterally administered vitamin B_{12} with a maximum reticulocyte response of 30 per cent in seven days.

In order to establish that this megaloblastic anemia was due to dietary deficiency of vitamin B_{12}, these investigators studied the patient's absorption of cobalt-labeled (Co^{60}) vitamin B_{12}. It was shown that the labeled vitamin B_{12} appearing in the feces after an oral dose was 37 per cent, well within the normal range. Furthermore, the gastric juice from this individual permitted a patient with pernicious anemia to utilize orally administered Co^{60}-labeled vitamin B_{12} in a normal manner. The deposition of Co^{60} in the liver and the urinary excretion of Co^{60}-labeled vitamin B_{12} were also within the normal range. These tests demonstrate that this malnourished patient secreted adequate amounts of intrinsic factor. It is noteworthy that the development of these tests cited above permitted the diagnosis in this patient although he was receiving vitamin B_{12}, an achievement necessarily impossible before isotopic labeling methods.

The authors speculate about the rarity of this type of pernicious anemia, as malnutrition is widespread throughout the world. At least four factors contribute to the rarity of this condition. First, only 10 to 15 per cent of the average daily intake of vitamin B_{12} is necessary to prevent a deficiency state. Achievement of such a deficiency would mean a drastic reduction in all protein and unrefined grain products. Second, the deficiency must be pure without evoking other dietary deficiencies. Third, when these conditions are met, they have to be satisfied for at least two years. Finally, there must be no access to the numerous multivitamin preparations. In areas of the world where medicinal vitamin supplements are not readily available, diagnostic facilities may also be lacking.

Almost simultaneously with the appearance of this report, R. J. Harrison, C. C. Booth, and D. L. Mollin (*Lancet* **I,** *727 (1956)*) have discussed a similar experience. Their patient was a 77 year old vegan (one who eats only foods of vegetable origin) who, for thirteen years, had lived on bread, margarine, tea and potatoes. He entered the hospital with a typical picture of untreated pernicious anemia. His hemoglobin was 5.4 g. per cent and the red cell count was 1.2 million. The bone marrow and blood smear appearances were compatible with pernicious anemia. The serum vitamin B_{12} level was 77 micrograms per cent.

In this patient a normal absorption of orally administered vitamin B_{12} was demonstrated. At the dosage used, a normal hematologic response would not have been anticipated in the absence of intrinsic factor. Thus, the presence of intrinsic factor was inferentially demonstrated and the nutritional basis of the pernicious anemia was shown to be probable.

These two cases are of interest in that they support Castle's original hypothesis that a deficiency of either food factor or gastric factor could lead to pernicious anemia. This demonstration, however, in no way sanctions the indiscriminate use of orally administered vitamin B_{12}.

ALKAPTONURIC ARTHRITIS

Alkaptonuria is an "inborn error of metabolism" in which the subjects have a defect in the metabolism of the aromatic amino acids phenylalanine and tyrosine (*Nutrition Reviews* **10,** *263 (1952)*). These individuals excrete homogentisic acid in the urine, which on oxidation gives the urine its characteristic dark color. In infants this aberration usually is an inconvenience because of staining of the diapers. The possibility also occurs that the condition may be faultily diagnosed as glycosuria due to the strong reducing properties of the homogentisic acid.

Later on in life there is usually ochronosis, a deposition of pigment in various tissues. This is first noted in the cartilage of the ear and nose and also as brown patches in the sclera. S. Sacks (*J. Bone Joint Surg.* **33B,** *407 (1951)*) reported that in 50 per cent of the cases of alkaptonuria reported there was evidence of ochronosis and degenerative arthritis. The joint involvement occurs in the older age groups. H. Milch and R. A. Milch (*J. Internat. Coll. Surg.* **15,** *669 (1951)*) reported 4 cases of alkaptonuria, 2 of which were in individuals under 21 years of age who showed no increased calcification of the spine or joint changes on X-ray examination. Of the 2 older patients (54 and 60 years), one had increased calcifications in the intervertebral disks and the other had osteoarthritis of the right hip.

More recently A. J. Harrold (*J. Bone Joint Surg.* **38B,** *532 (1956)*) has studied 2 cases of alkaptonuric arthritis. Both individuals had been known to have had alkaptonuria for many years. One patient was 53 years old and had arthritis of the spine which showed the characteristic X-ray picture of alkaptonuric arthritis. Roentgenograms of the spine of such patients show the intervertebral disk as a dense calcified wafer-like structure between the vertebral bodies. In alkaptonuric arthritis the primary changes are in the intervertebral disks rather than in the ligaments or interarticular joints. Thus there seems to be a triad: alkaptonuria, ochronosis and arthritis. The explanation of why an error in tyrosine and phenylalanine metabolism should affect the metabolism of bone is not readily available. Also the fact that there is increased pigment deposition in the bone and other tissues does not clarify the problem of the deranged bone metabolism.

DIETARY FAT AND PLASMA CHOLESTEROL

The complex relationships existing between the diet and the blood lipids on the one hand and between these factors and the development of atheromatous lesions on the other have invited the investigative efforts of many laboratories but have so far resisted all efforts toward the formulation of a consistent explanation. Thus possible differences in the effects on blood lipids of dietary fat of animal or vegetable origin have been investigated by several groups with little agreement in the results (*Nutrition Reviews* **13,** *8, 44, 138 (1955)*). Some obvious causes have combined to produce this state of affairs. In some cases, little attention has been paid to the pre-experimental diet or its effect on the plasma lipids. Evidently, if these were already abnormal, an abnormal response to a change in diet might be expected. In other cases, the experimental diet has not been carefully formulated or well analyzed, and the subjects may have lost or gained weight during the experiment, conditions which are known to affect the plasma lipids. Finally, it is possible that the proper questions were not asked, leaving little hope that satisfactory answers would be forthcoming.

Recently, J. M. R. Beveridge and co-

workers (*J. Nutrition* **56,** *311* (*1955*); *Nutrition Reviews* **14,** *107* (*1956*)), using adequate numbers of healthy medical students as experimental subjects and a well controlled homogeneous diet, confirmed the findings of E. H. Ahrens, D. H. Blankenhorn, and T. T. Tsaltas (*Proc. Soc. Exp. Biol. Med.* **86,** *872* (*1954*)), L. W. Kinsell *et al.* (*J. Clin. Nutrition* **1,** *224* (*1953*)) and others that a diet in which the fat was of vegetable origin resulted in a lowering of plasma cholesterol levels while one in which animal fat was included had the opposite effect. A previous experiment (G. A. Mayer, W. F. Connell, M. S. DeWolfe, and Beveridge, *Am. J. Clin. Nutrition* **2,** *316* (*1954*)) in which increases in plasma cholesterol levels had been obtained with both types of fat was explained by the fact that some material derived from animal fat was present in all diets and may have acted in conjunction with the vegetable oil to raise the plasma cholesterol. Several questions remain to be answered, however. First, is a factor in vegetable fat responsible for lowering plasma cholesterol? Second, does a factor in animal fat cause an increase? Third, if in either case the answer is in the affirmative, can the factor be identified?

To attempt to answer these and other questions, J. M. R. Beveridge, W. F. Connell, and G. A. Mayer (*Canad. J. Biochem. Physiol.* **34,** *441* (*1956*)) performed three experiments using a carefully defined homogeneous diet and adequate numbers of medical students as volunteer subjects. Care was taken in all experiments to allow the subjects to adjust to the basal diet before the experimental diets were introduced, and to make sure that all subjects maintained body weight during the experiments.

In the first experiment, the subjects were given diets in which 58.5 per cent of the diet calories were from fat. The previous study was confirmed, since the group on the corn oil-containing diet showed significant decreases in plasma cholesterol during eight days and a further slight decrease after sixteen days. When butterfat was substituted for corn oil after eight days, a significant rise in plasma cholesterol occurred during the second eight-day period. Lard also produced a significant increase, while chicken fat and beef dripping produced smaller increases.

In the second experiment, the subjects were maintained on a "fat-free" diet (0.7 per cent of the calories as fat) for eight days and then given diets containing butterfat or corn oil at 20 or 60 per cent dietary levels. Average plasma cholesterol was lowered about 22 per cent on the "fat-free" diet. Those groups which were then given 20 and 60 per cent corn oil in the diet showed further decreases of 7 and 15 per cent respectively, while the same amounts of butterfat produced increases of 7 and 22 per cent.

In the third experiment, the high butterfat diet was followed by diets in which 25, 50, 75 and 100 per cent of the butterfat was replaced by corn oil. The effect of the 25 per cent corn oil mixture was similar to that of butterfat. All the other mixtures, however, brought about decreases in plasma cholesterol roughly proportional to their percentage of corn oil. In all the experiments, the cholesterol levels of subjects after eight days on the high butterfat diet were no greater than they had been previously on a free choice diet (averaging about 45 per cent fat).

It seems possible that dietary substances other than animal fat can normally raise the plasma cholesterol concentration or, perhaps, that individual maxima for plasma cholesterol had already been reached.

These studies have apparently answered two of the original questions. It was evident that all the animal fats tested contained substances which acted to increase plasma cholesterol, butterfat having the greatest effect, followed by lard. It was equally evident that the corn oil contained substances which brought about a lowering of the plasma cholesterol, since the corn oil-containing diets were more effective in this respect than the "fat-free" diet.

The answer to the third question concerning the nature of either type of substance

was not forthcoming from these experiments. It may be recalled, however, that butterfat and lard have been found to be the most effective fats in promoting rapid growth and at the same time decreasing life-span of rats (H. J. Thomasson, *J. Nutrition* **57,** *17* (*1955*); *Nutrition Reviews* **14,** *349* (*1956*)). It is possible that for both effects readily digestible short-chain saturated acids are concerned. In the case of the vegetable oils, several possibilities present themselves. It is unlikely that the plant sterols or the lack of dietary cholesterol produces this effect since these factors have not been shown to be effective in man. The essential fatty acids, or perhaps their ratio to the saturated fatty acids, are likely suspects since these have been implicated in cholesterol transport (R. B. Alfin-Slater, L. Aftergood, Arthur F. Wells, and H. J. Deuel, *Arch. Biochem. Biophys.* **52,** *180* (*1954*)). In this connection it should be noted that chicken fat is comparatively rich in the essential fatty acids. Finally, it is possible, in view of the work of G. Weitzel, H. Schon, F. Gerz, and E. Buddecke (*Z. f. physiol. chem.* **304,** *247* (*1956*)), that the fat-soluble vitamins A and E are concerned in the lowering of plasma cholesterol concentration as well as in the reversal of atheromatous plaque formation.

EFFECT OF VITAMIN SUPPLEMENTS DURING PREGNANCY ON THE INTELLIGENCE OF OFFSPRING

The experimental studies of Warkany and others have indicated that congenital anomalies can be induced in animals by dietary deficiencies during pregnancy. The difficulty in ascribing to a dietary factor the production of human congenital anomalies has been discussed (*Nutrition Reviews* **13,** *289* (*1955*)). Malnutrition in the mother has been reported to predispose to stillbirths, neonatal deaths and premature births, although the observations of several authors in this field are conflicting (*Ibid.* **12,** *260* (*1954*)).

The effect of maternal diet on the development of the nervous system and the intelligence of offspring is a problem of great importance. To gain information on this topic a study has been conducted by R. F. Harrell, E. R. Woodyard, and A. I. Gates (*Metabolism* **5,** *555* (*1956*)), on a group of 2400 pregnant women.

Half of these women were studied in Norfolk, Virginia. They were predominantly Negro and lived under crowded urban conditions. Because of the low economic status the diets were said to be "poor." The remaining women were from a rural area of Leslie County, Kentucky, and their diet was said to be plain but not conspicuously inadequate. The women were assigned to one of four groups by rotation and received one of the following supplements: (A) 200 mg. of ascorbic acid, (B) 2 mg. of thiamine, 4 mg. of riboflavin, 20 mg. of niacinamide, 15 mg. of iron, (C) inert placebo, (D) 2 mg. of thiamine. Neither the investigators nor the patients knew the contents of the supplement.

When the offspring of the Norfolk mothers were 3 years old, and again when they were 4, they were subjected to intelligence testing using a modified Stanford-Binet scale. About one-half the women in the Norfolk group cooperated sufficiently to take the supplements and only 518 children could be located for testing at 3 years and 370 children at 4 years of age.

It is interesting that the birth weights of the children and the clinical course of the pregnancies of the four groups were not significantly different.

The results of the intelligence tests of the offspring from the four groups were compared using the analysis of variance technic. The children of mothers receiving the placebo had the lowest mean intelligence and although the children from mothers receiving

the multiple supplement tablets (group B) had the highest mean intelligence, this group did not differ from groups A and D in which ascorbic acid and thiamine were given separately. When the tests were repeated the following year the difference between the placebo group and the group receiving the mixed vitamin supplement amounted to 8.1 I.Q. points which, although small, is educationally significant.

Intelligence quotients were determined in 811 3 year old children of mothers receiving supplements in Leslie County, Kentucky. The mean intelligence of all groups was higher than in Norfolk and there was no significant difference between the groups receiving different supplements.

Despite the suggestive evidence that the nutritional supplements resulted in improved intelligence in the offspring of the Norfolk mothers, this study points up the frustrations which beset the nutritional investigator in this field. Accurate dietary histories could not be obtained in the Norfolk group because of the tendency to "gross fabrication" and in the Kentucky mountain folk because of their "extraordinary individual reticence." The investigators were also unable to start supplementation at a uniform time in pregnancy and some women received tablets for only short periods. It would seem unlikely that a supplement given for such a short period could exert the same benefit as one given for a longer period. The authors made a considerable effort to locate the children in Norfolk for intelligence testing but nevertheless considerably less than half could be found due to the migratory nature of this element of the American population. It is hard to predict if this selection introduced any bias.

The statistical analysis of the data can be commended because the analysis of variance technic is a powerful statistical tool for evaluating the results of such a study as the one carried out in Norfolk. The design of the experiment is open to one criticism: the assignment of supplementation by rotation of cases as done in this study is not so satisfactory as using a system for true randomization.

It is apparent that this ambitious study of supplementation during pregnancy leaves the question of its effects undecided. Investigation in a more stable locale where the experimental conditions could be controlled more carefully will be necessary before any positive conclusions can be drawn as to the role of vitamin supplements during pregnancy on the intelligence of the offspring.

CARBOHYDRATE METABOLISM DURING HYPOTHERMIA

The increasing use of hypothermia (lowered body temperature) during extensive operations on the cardiovascular system has created a series of new problems for the physician. V. Wynn (*Lancet* **II,** *575 (1954)*) has reported studies of the alterations in glucose metabolism which occur during hypothermia.

The patient observed was undergoing extensive vascular surgery, and during the first nine hours of a twelve-hour procedure received 1300 ml. of 5 per cent glucose in water as well as blood replacement. At four and one-half and nine hours after the start of surgery the plasma sodium dropped to 123 milliequivalents per liter and the serum potassium decreased to 2.75 milliequivalents per liter. The plasma glucose reached a level of 1040 mg. per cent. Normally, this amount of infused glucose would be metabolized and the excess water either excreted or distributed throughout the total body water. A similar amount of glucose and water given to a patient undergoing comparable surgery without hypothermia was metabolized without difficulty.

Preliminary calculations from the total glucose infused and the change in plasma concentration showed the glucose to be distributed in approximately 7.7 liters. This

was 16 per cent of the patient's weight, a reasonable value for the volume of extracellular fluid. In spite of a number of other considerations, the presumption is clear that the excess glucose was not transported intracellularly but, remaining in the extracellular compartment, caused the expansion of this compartment by intracellular water and the consequent lowering of serum sodium.

This interpretation was put to an examination by administering glucose tolerance tests to dogs under anesthesia and hypothermia. With anesthesia alone, no significant rise in plasma glucose was noted. The addition of hypothermia caused a variable rise in plasma glucose. However, when 25 g. of glucose were given intravenously, the plasma glucose rose as high as 622 mg. per cent, and it was calculated that only 30 per cent of this glucose was metabolized in two and one-half hours. One dog received 25 units of regular insulin before the glucose administration and showed a rise in blood sugar of only 225 mg. per cent. The volume of distribution of glucose thirty minutes after injection was in agreement with the calculated extracellular fluid volume. The fall in serum sodium at thirty minutes (7.5 to 11.5 mEq per liter) was also in agreement with the calculated value.

Since the total body water was not increased throughout the study, the dilution of the serum sodium must have resulted from the transfer of intracellular water. Thus, the original hypothesis was amply verified and the dangers of the intravenous administration of glucose during hypothermia were made apparent.

Since fructose is metabolized somewhat differently than glucose, and since insulin is less important in the dissimilation of fructose than glucose, V. Wynn (*Clin. Sci.* **15,** *297 (1956)*) investigated its metabolism during hypothermia. He studied 4 patients undergoing extensive vascular surgery under hypothermia as well as a control group in which hypothermia was not used. Intravenous fructose tolerance tests and changes in plasma potassium and phosphorus were observed.

Fructose tolerance was markedly impaired by hypothermia, with the thirty-minute plasma levels averaging two and one-half times those of the control group. Since hepatectomy in the dog and severe hepatic disease in man have little effect upon the distribution of fructose throughout the total body water, it is unlikely that these effects during hypothermia were due to liver dysfunction. Hypothermia may interfere with fructose transport across cell membranes, as it does with glucose.

Ninety minutes following the injection of the fructose plasma fructose levels were low, but there was an increment in plasma glucose between 135 mg. per cent and 189 mg. per cent. These increases in plasma glucose could be explained by the conversion of 42 to 80 per cent of the fructose to glucose.

In the control group the serum potassium fell and remained below the preinjection level for two hours. During hypothermia the serum potassium level rose for the first sixty minutes and then fell to levels below that of the preinjection level. There was no obvious explanation for the initial rise in serum potassium.

The results of these two papers demonstrate important defects in the metabolism of exogenous and endogenous glucose and fructose during hypothermia. The details of these defects have not been worked out and should provide a fertile field for future investigation.

AMINO ACID SUPPLEMENTATION OF CASEIN DIETS FOR CHICKS

It is well known that casein is deficient in certain amino acids. H. J. Almquist, E. Mecchi, and F. H. Kratzer (*J. Biol. Chem.* **141,** *365 (1941)*) reported that the addition of glycine, arginine, or creatine to a casein diet for chicks improved growth and resulted

in increased concentrations of muscle creatine. These results and others of a similar nature led to the designation of glycine as an essential amino acid for the chick (Almquist and C. R. Grau, *J. Nutrition* **28,** *325 (1944)*). More recently it has been shown that the glycine requirement of the chick can be replaced by serine (R. L. Wixom, G. E. Pipkin, and P. L. Day, *Ibid.* **56,** *409 (1955)*). A. W. Wietlake, A. G. Hogan, B. L. O'Dell, and H. L. Kempster (*Ibid.* **52,** *311 (1954)*) reported that for chick growth casein is grossly deficient in arginine.

The effects of supplementation of casein diets with amino acids on growth and muscle creatine concentration of chicks have been reported by H. Fisher, R. C. Salander, and M. W. Taylor (*J. Nutrition* **58,** *459 (1956)*). Chicks were fed a basal diet of 25 per cent casein with corn oil, cerelose, vitamins and minerals. Various groups were given supplements of 1 per cent arginine, 2 per cent glycine, and 0.3 per cent methionine separately and in all possible combinations. Another group was given a supplement of 0.8 per cent creatine, and control animals were given a ration of natural foodstuffs.

Of the single supplements tested only arginine gave a significant growth response. The addition of glycine to the arginine-supplemented diet resulted in further improvement in growth rate. Chicks receiving this diet grew as well as those fed the practical ration. The addition of glycine, methionine, or creatine to the basal diet did not increase the growth rate of the chicks.

The only supplements which led to significant increases in muscle creatine concentration were arginine plus glycine and creatine.

From the results of these studies and others reported in the literature it is quite clear that chicks fed casein as the protein source require additional arginine for maximum growth. The situation with other amino acids is not so clear-cut. The most glaring discrepancies concern the effects of supplementary glycine and creatine. The divergent results which have been reported may be related to the level of protein in the diet (*Nutrition Reviews* **8,** *163 (1950)*), or to some more subtle difference in the basal diets.

FOLACIN IN REPRODUCTION OF TURKEYS

The importance of folacin in the normal reproduction of chickens and rats is well known (*Nutrition Reviews* **8,** *260, 303 (1950)*; **9,** *12 (1951)*; **11,** *121 (1953)*). However, few studies have been done in turkeys with this nutrient. F. H. Kratzer, P. N. Davis, and U. K. Abbott (*Poultry Sci.* **35,** *711 (1956)*) have recently studied the effects of folacin deficiency in turkey breeding hens. A practical ration composed of folacin-low ingredients (corn, wheat, fish meal, casein, minerals and vitamins) was used in two experiments. No antagonist was used to enhance the deficiency.

The first sign of deficiency was a decrease in hatchability of eggs which was observed after the hens were on the experiment fifty days. By ninety days hatchability dropped to 10 per cent or lower. Abnormal embryos were observed in eggs from deficient hens, as evidenced by twisting of the legs, enlargement of the hock joint, micromelia (small limbs), hemorrhage, and edema. All abnormal symptoms were prevented by the addition of folacin to the ration. Several levels were tested and it was concluded that a total of 0.7 mg. of folacin per kilogram of ration was necessary to give maximum hatchability (60 to 70 per cent) in the presence of ample vitamin E. No gross abnormalities were seen in the deficient hens for the twenty-three-week period and there was no effect on egg production.

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Pearson, and R. M. Sherwood (*J. Nutrition* **35,** *89* (*1948*)) could not find any effect of folacin on hatchability of eggs from turkeys fed a practical ration. However, their birds were raised in breeding pens and were not on wire floors as were the birds of Kratzer and co-workers. It is very possible that consumption of droppings (which are known to contain folacin) could account for the differences in results of the two groups. The authors did find that poults from folacin-deficient turkeys did not grow so well as poults from normal hens.

These studies, showing the need for folacin by turkeys for the prevention of various abnormalities of the embryo in the hatching egg, do more than point out the possible importance of this vitamin in practical turkey rations. They also demonstrate the availability of another species in which basic information can be gained on the role of folacin in reproduction.

PLANT STEROLS AND CHOLESTEROL ABSORPTION

It has been known for some time that dihydrocholesterol and plant sterols protect chicks against the atherosclerosis produced by a high cholesterol level in the ration (*Nutrition Reviews* **12,** *54* (*1954*)); D. W. Peterson (*Proc. Soc. Exp. Biol. Med.* **78,** *143* (*1951*)). More recent work suggests that the addition of plant sterols to a normal diet fed adult men who have high blood cholesterol levels is effective in lowering that level (*Nutrition Reviews* **14,** *39* (*1956*)). The mechanism whereby the plant sterols influence the cholesterol levels has been unknown.

A. C. Ivy, T. Lin, and E. Karvinen (*Am. J. Physiol.* **183,** *79* (*1955*)) suggest that a possible explanation for the action of plant sterols on cholesterol absorption may reside in a competition between animal and plant sterols for the absorptive mechanism in the intestinal wall. They were led to the problem by the lack of agreement in the literature as to whether plant sterols are actually absorbed. Most of the earlier work led to the conclusion that the plant sterols were not absorbed (*see also Nutrition Reviews* **14,** *285* (*1956*)).

Ivy and co-workers fed adult rats a ration composed of purified ingredients that were free of cholesterol. The feeding of 96 mg. of cholesterol (dissolved in oleic acid which was added to the day's feed allotment) per day to the rats fed this ration resulted in the absorption of 61 per cent of the sterol. The absorption was calculated on the basis of dietary intake and fecal excretion. The addition of 192 mg. of soybean sterol (93 per cent sitosterol) to the above reduced the absorption of cholesterol to 32 per cent. In the latter case, a correction was made for the fecal excretion of sterols which occurred when soybean sterols alone were added to the ration. Studies with dihydrocholesterol showed the same results.

On the basis of these results, Ivy and associates calculated that both soybean sterols and dihydrocholesterol at the levels used reduced the absorption of cholesterol by approximately 40 per cent.

Blood cholesterol levels indicated that some of the soybean sterols were absorbed when fed to the rats. The blood cholesterol level of rats on the basal diet containing no sterols was 68 mg. per cent. When 192 mg. of soybean sterols per day were fed, the "apparent" blood cholesterol level was 80 mg. per cent; when 96 mg. of cholesterol were fed, the level was 124 mg. per cent; when both cholesterol and soybean sterols were fed, the level was 117 mg. per cent. Making allowance for the "apparent" cholesterol blood level when the soybean sterols were fed, it appears that the level observed in the group receiving both soybean sterols and cholesterol presented additional evidence for

a reduction in the cholesterol absorption in the presence of soybean sterols.

Ivy and co-workers went on to show that the intestine of the rat has a limited capacity to absorb cholesterol. This conclusion was based on the observation that when different groups of rats were fed daily supplements of cholesterol amounting to 92, 192 and 288 mg., the absorption was 61, 47 and 32 per cent respectively. On this basis, they suggest that soybean sterols and dihydrocholesterol reduce the absorption of cholesterol by competing for the intestinal sterol absorptive mechanism.

Additional evidence for the absorption of the plant sterols was secured by R. G. Gould (*Circulation* **10,** *589 (1954)*). He fed both mixed sitosterols and pure sitosterol, which were labeled with tritium, to rats and human beings. About 0.1 to 1.0 per cent of the 400 mg. dose fed was absorbed in the human subjects. The disappearance rate of sitosterols from the blood was reported to be faster than that for labeled cholesterol. This suggested that sitosterol does not accumulate in the body, partly because of its rapid excretion. The tagged sitosterol was absorbed by the rats but not so efficiently as tagged cholesterol. Unfortunately, the brevity of the report does not permit an evaluation of the results obtained.

G. L. Curran and R. L. Costello (*Proc. Soc. Exp. Biol. Med.* **91,** *52 (1956)*) secured evidence that the plant sterols were absorbed by rabbits but not by rats. They found that when adult rabbits fed a commercial ration were given by mouth 9 g. of soybean sterols a week there was no demonstrable plant sterol in the serum. The livers of these animals, however, contained from 3 to 4 mg. of plant sterols per gram of liver. Since 36 g. of soybean sterols were fed over the four-week period, it is obvious that a relatively small amount of these sterols was absorbed by the rabbits.

Autopsy showed that all of the rabbits receiving the soybean sterol supplement had atheromatous lesions of the aorta at autopsy. The presence of soybean sterols in the lesions was shown by infrared absorption spectra. This indicates that the plant sterols are not only absorbed by the rabbit as well as by chickens (C. W. Nichols, S. Lindsay, and I. L. Chaikoff, *Proc. Soc. Exp. Biol. Med.* **89,** *609 (1955)*), but that they are also atherogenic.

According to the above work, the rat showed no measurable amount of soybean sterols in the liver when 3 per cent of these sterols were added to a commercial ration. The presence of the plant sterols in the ration reduced the liver sterol levels during the first two weeks of the experiment. However, this was associated with an increased rate of sterol synthesis in the liver as shown by the uptake of C^{14}-labeled acetate by liver slices.

It is likely that the earlier work which led to the conclusion that no plant sterols were absorbed by either rabbits or rats may be explained partly on the basis of technic and the short period of dietary supplementation. R. Schoenheimer (*Science* **74,** *579 (1931)*) examined the thoracic lymph of rats and rabbits fed plant sterols and was unable to detect any of these sterols in the lymph. Perhaps if tagged sterols had been available to him, a different answer would have been secured. It is difficult to know why Schoenheimer's rabbits fed large amounts of sitosterol over long periods of time did not develop atheromatous lesions (Schoenheimer and D. Yuasa, *Z. f. physiol. Chem.* **180,** *5 (1929)*).

Increasing evidence is accumulating which indicates that the plant sterols are absorbed by both men and animals. They are, however, absorbed to a much smaller degree than is cholesterol. There is a possibility that the action of the plant sterols in reducing the blood cholesterol levels may be associated with a competition of the plant and animal sterols for the available absorptive capacity of the intestinal tract.

NUTRITION AND BACTERIAL INFECTION

Much has been written concerning the possible relationships between nutrition and resistance or susceptibility to infectious agents. Present evidence indicates that certain B-vitamins, notably pyridoxine, pantothenic acid and folacin, play a significant role in antibody synthesis (*Nutrition Reviews* **10,** *353* (*1952*)). Antibody production is diminished in severe protein deficiency (*Ibid.* **3,** *86* (*1945*); **6,** *234* (*1948*)). J. Seronde, T. F. Zucker, and L. M. Zucker (*J. Nutrition* **59,** *287*, *299* (*1956*)) have attempted to evaluate the relationship between the resistance of animals to infection and antibody production as influenced by diet.

In earlier work it was established that young pantothenic acid-deficient rats spontaneously developed infections with the *Corynebacterium* (strain 197) (T. F. Zucker and L. M. Zucker, *Proc. Soc. Exp. Biol. Med.* **85,** *517* (*1954*)). The *Corynebacterium* isolated from lesions of such infected animals produced the disease when inoculated into other pantothenic acid-deficient rats, but rats on complete diets, natural or purified, were resistant to infection under these conditions (J. Seronde, *Ibid.* **85,** *521* (*1954*)).

Further studies revealed that the susceptibility to infection by the bacterium increased steadily with time on the pantothenic acid-deficient diet over a period of ten to forty days and that the susceptibility could be induced after a longer period in rats on a diet only partially deficient in pantothenic acid (J. Seronde, L. M. Zucker, and T. F. Zucker, *J. Inf. Dis.* **97,** *35* (*1955*); L. M. Zucker, Seronde, and T. F. Zucker, *Arch. Biochem. Biophys.* **55,** *9* (*1955*)).

In the recent studies reported these workers used a diet composed of the following parts: vitamin-free casein, 18; L-cystine, 0.2; salt mixture, 3.7; cottonseed oil, carrying supplements of vitamins A and E, 5; cellulose, carrying supplements of vitamins B and K, 1; DL-cysteine hydrochloride (as antioxidant), 0.05; and glucose to equal 100. Diminution or omission of pyridoxine, thiamine, or pantothenic acid permitted a study of the influence of these nutrients on the susceptibility to infection.

The infection source consisted of a master culture of *Corynebacterium kutscheri* and the dose consisted of 0.15 ml. of a twenty-four-hour broth culture given intraperitoneally. In addition to inoculation studies, spontaneous infection was investigated by interspersing cages of noninoculated animals with those of animals that had received *Corynebacterium* inoculations.

Spontaneous *Corynebacterium* infections were widespread in animals that were placed on the pantothenic acid-deficient diet at 3 to 4 weeks of age. In contrast, no infections were seen in comparable animals on the complete diet, or on diets deficient in pyridoxine or partially deficient in thiamine. Strain differences were noted on the pantothenic acid-deficient diet. An infection incidence of over 50 per cent was noted in 13C and 14C strains of rats, while in the 9B strain the infection incidence was about 10 per cent. Once recognized, the infectious condition is reported to be easily distinguished from other known respiratory diseases of rats. Free serofibrinous exudate, opaque white nodules and adhesions are characteristic of thoracic lesions.

The inoculation experiments likewise revealed a high susceptibility of the pantothenic acid-deficient rat to infection with *Corynebacterium*. A 100 per cent infection incidence was noted in pantothenic acid-deficient rats of strain 13C, and all animals died of the infection in one to eleven days. Pantothenic acid-deficient rats of the 9B strain appeared to be more resistant, with only 3 of 11 developing severe infections. Approximately this same level of infection

was seen in animals of the 13C strain on diets deficient in pyridoxine or partially deficient in thiamine. Only one of 43 inoculated rats fed the complete diet exhibited infection. When the caloric consumption of the complete diet was restricted to the level consumed by rats on the deficient diets, the response was about the same as it was to the complete diet fed ad libitum.

Thus it is indicated that a complete diet protected young rats against serious infection from the *Corynebacterium* and that the barrier to infection is dependent upon an adequate pantothenic acid intake.

The marked difference in behavior of the two strains of rats with regard to resistance to infection under a pantothenic acid-deficient regimen offers a challenge to the simple concept of a blanket minimum daily requirement of a nutrient for a given species.

It was evident from agglutination titers following vaccination with a formaldehyde-killed culture of *Corynebacterium* that pyridoxine deficiency entirely suppressed agglutinin production, while pantothenic acid deficiency seriously inhibited it, without complete suppression. Thiamine deficiency did not significantly depress antibody response (agglutinin production). The agglutinin responses to vaccine of *Corynebacterium* strain 197 in the three deficiencies are similar to those recorded by J. Pruzansky and A. E. Axelrod (*Proc. Soc. Exp. Biol. Med.* **89,** *323 (1955)*) for purified toxoid of *Corynebacterium diphtheriae*.

The wide difference of behavior of pyridoxine-deficient and thiamine-deficient rats with respect to antibody response to the vaccine and the equal resistance of such rats to *Corynebacterium* (strain 197) infection thus indicates that under these conditions the ability to produce antibodies has little relation to resistance to natural infection.

These results again emphasize the complexity of the problem of diet and susceptibility or resistance of animals to bacterial invasions. Since antibody response, as typified by agglutinins, has been ruled out as a major factor in resistance to *Corynebacterium* in the rat, the authors recognize the many biochemical determinants that may be involved in which pantothenic acid might play a role.

A failure to correlate resistance to infection with antibody response should not be considered unusual since it has been emphasized that the microbial diseases for which immunity mechanisms are not known far outnumber those for which resistance can be explained in terms of recognized immunologic reactions (R. J. Dubos, "Biochemical Determinants of Microbial Disease," *Harvard University Press, Cambridge (1954)*).

COMPARISON OF LIPOTROPTIC AGENTS

In few fields does the choice of a basic experimental diet influence the course of the experiment more than in studies of lipotropic agents. Thus, if the diet is inadequate in certain factors, such as the methyl transferring agents, vitamin B_{12} or folacin, methionine may be inactive. On the other hand, when the diet is made optimal for growth, amounts of lipotropic agents which are adequate for a lower growth rate become insufficient, and an increase in liver fat results (D. J. Mulford and W. H. Griffith, *J. Nutrition* **23,** *91 (1942)*; *Nutrition Reviews* **1,** *168 (1943)*). It is evident that between the extremes of inadequate and optimal diets lies a complete spectrum of diets with varying effects on liver fat infiltration and on the action of different lipotropic agents.

In an attempt to compare the potency of the three main lipotropic agents, choline, betaine and methionine, C. H. Best, C. C. Lucas, J. H. Ridout, and J. M. Patterson (*J. Biol. Chem.* **186,** *317 (1950)*) used a diet with sufficient protein only to maintain

weight in order to prevent the effect of growth in increasing the requirements of methyl donors. Since this diet was also inadvertently low in the newly recognized vitamin B_{12}, the validity of the results was later questioned by the authors.

Consequently an experiment was designed (R. J. Young, C. C. Lucas, J. M. Patterson, and C. H. Best, *Canad. J. Biochem. Physiol.* **34,** *713 (1956)*) in which weanling rats were allowed to grow on a diet containing the minimum amount of protein necessary to support optimal growth when supplemented with lipotropic substances. In this case the efficiency of the three substances could be compared in their effect on growth rate, liver fat and renal lesions. Since it had been shown in the previous experiment that the relative effectiveness of lipotropic agents differs widely for different dose ranges, complete dose-response curves were prepared for each substance.

A basal diet was developed which supported optimal growth when supplemented with adequate choline or betaine. The per cent composition of the diet was as follows: alcohol extracted peanut meal (12), soya protein (8), casein (1), L (−) cystine (0.2), salt mixture (3), celluflour (1), corn starch (10), dextrin (10), sucrose (38.8), hydrogenated fat (10), corn oil (5), sucrose-vitamin mixture (1), cod liver oil concentrate and d-alpha-tocopheryl acetate (0.01 each). The essential amino acids were apparently supplied adequately by the total amount of protein (15 per cent) in the diet since additional lysine, histidine, or threonine did not improve the growth rate. The rats were fed this basal diet ad libitum either unsupplemented or with varying amounts of choline, betaine, or methionine. After twenty-one days, the survivors were killed and all animals were compared with respect to growth rate, food consumption, liver fat and renal lesions.

The basal diet led to high liver fat and kidney damage. The choline supplement was effective at the following concentrations: protection against renal lesions, 0.04 per cent; maximum growth rate, 0.10 per cent; normal liver fat, 0.12 to 0.16 per cent. Under the same conditions, the concentrations of betaine which were equally effective for the three criteria were: 0.20 to 0.24; 0.24 to 0.28; and 0.32 per cent, respectively. Thus on a molar basis (the molecular weights are nearly the same) choline was about three times as effective as betaine from normal to 27 per cent liver fat. These results confirm the previous findings of D. J. Mulford and W. H. Griffith (*J. Am. Chem. Soc.* **63,** *939 (1941)*).

Methionine, on the other hand, although its effects were comparable to betaine at low levels on a molar basis, was progressively less effective at increasing concentrations; a normal liver fat content could not be achieved with methionine either with or without an additional 0.2 per cent cystine.

These experiments confirm previous results showing that choline is about three times as efficient as betaine as a lipotropic agent if measurement is made in the range of normal to 27 per cent liver fat. On other portions of the dose response curve the ratio may vary considerably and consequently is not a useful comparison. Reasons for this difference in efficiency have been advanced. For instance, it has been equated to the knowledge that only one of the three methyl groups of betaine is available for transmethylation. However, the multiple nature of the functions of these compounds may make the exact correspondence of the ratios purely fortuitous. In the same light, it is difficult to explain the inability of methionine to replace choline completely, even at very large doses (*see also Nutrition Reviews* **2,** *358, (1944)*). It is evident, however, that both compounds have some functions which are similar (such as transmethylation) and some which are distinct (such as lipotropism). The ability of one compound to replace another in one function does not necessarily imply the ability to replace it completely in all functions.

MECHANISM OF LECITHIN BIOSYNTHESIS

Choline serves many useful functions in the animal body. The methyl groups may be transferred to participate in the synthesis of various metabolites, or the intact molecule may be utilized in synthetic processes. One important example of the latter type of function is the synthesis of the phospholipid lecithin. A striking sign of choline deficiency in animals is fatty infiltration of the liver and the lipotropic effect of choline is due to the fact that it serves as a precursor of lecithin.

The biochemical steps in lecithin biosynthesis have been studied and certain details are established. Choline is first phosphorylated by adenosine triphosphate (ATP) to form phosphorylcholine. It has been shown with the aid of phosphorylcholine doubly labeled with radioactive phosphorus and carbon (P^{32} and C^{14}) that the intact molecule is incorporated into lecithin (A. Kornberg and W. E. Pricer, *Fed. Proc.* **11,** *242 (1952)*; M. Rodbell and D. J. Hanahan, *J. Biol. Chem.* **214,** *607 (1955)*).

The intermediate steps in the synthesis of lecithin from phosphorylcholine have been investigated by E. P. Kennedy and S. B. Weiss (*J. Biol. Chem.* **222,** *193 (1956)*). The results are of particular interest because they demonstrate a function for another nucleotide, cytidine triphosphate (CTP). Phosphorylcholine labeled with P^{32} was incubated with a mitochondrial fraction of rat liver and lecithin was isolated after the incubation period. When the system contained a crude source of ATP the phosphorylcholine was rapidly incorporated into lecithin. However, when the ATP was replaced with adenosine monophosphate and an oxidizable substrate which should generate ATP, no appreciable quantities of labeled lecithin were produced. Also, when a rigidly purified sample of ATP was used, phosphorylcholine was not incorporated into lecithin.

These findings suggested that an impurity in the original ATP preparation was responsible for its activity. Accordingly a number of nucleotides were tested in the system. It was found that catalytic quantities of CTP would permit maximum activity of the system. None of the other nucleotides was active.

Attention was next directed to the mechanism of the CTP effect. The possibility that CTP reacted with phosphorylcholine to form cytidine diphosphate choline (CDP-choline) was considered. This compound was synthesized labeled with C^{14} in the ethanol moiety of the choline. It was observed that CDP-choline was incorporated into lecithin at a rapid rate by the liver mitochondria. The rate of incorporation of this compound was considerably greater than the rate of incorporation of the same amount of phosphorylcholine and CTP. Other experiments demonstrated that CDP-ethanolamine was extensively incorporated into the cephalin fraction of rat liver, indicating that CTP is a coenzyme in the formation of both major types of phospholipids.

The natural occurrence of CDP-choline was next investigated. Livers from rats and from laying hens were fractionated by chromatographic procedure and their CDP-choline concentration was estimated by an isotope dilution technic. Appreciable quantities were found, and the livers from laying hens contained approximately ten times as much of the nucleotide as did rat livers. This is of interest in view of the high rate of lecithin synthesis by laying hens and lends additional support for the role of CDP-choline as an intermediate in phospholipid synthesis.

In other experiments the enzymatic synthesis of CDP-choline and CDP-ethanolamine were studied. The incubation of a variety of rat tissues with phosphorylcholine or phosphorylethanolamine and CTP led to the synthesis of CDP-choline or CDP-ethanolamine. Thus enzymes were demonstrated for the formation of the CDP intermediate and

for its subsequent incorporation into phospholipids.

The lecithin molecule contains the diglyceride component in addition to its phosphoryl choline. The nature of the diglyceride fragment which accepted the CTP-choline fraction was studied. The synthesis of lecithin by normal mitochondria was not affected by the addition of diglyceride. It was felt that this result indicated adequate endogenous sources. When the mitochondria were treated with surface active agents the diglycerides were dissociated, and under these conditions the addition of synthetic alpha, beta-diglyceride markedly stimulated the synthesis of lecithin.

Lecithin biosynthesis appears to proceed by the following steps:

choline + ATP → phosphorylcholine
phosphorylcholine + cytidine triphosphate → cytidine diphosphate choline
cytidine diphosphate choline + alpha, beta-diglyceride → lecithin

With these results demonstrating a coenzyme role for CTP, it has been shown that the entire spectrum of nucleotides from ribonucleic acid possess coenzyme functions. Guanosine triphosphate is concerned in the incorporation of amino acids into protein (E. B. Keller and P. C. Zamecnik, *J. Biol. Chem.* **221,** *45* (*1956*)). Uridine diphosphate is involved in several metabolic reactions of carbohydrates (R. Caputto, L. F. Leloir, C. E. Cardini, and A. C. Paladini, *Ibid.* **184,** *333* (*1950*)). The numerous catalytic roles for ATP are well known.

PRECURSORS OF FOLACIN FACTORS IN BLOOD

Although many forms of folacin have been recognized, isolated and characterized, only fragmentary information exists about the nature of the native, or intact, forms of this vitamin as it occurs in animal tissues. In natural materials, the bulk of the folacin derivatives exist as bound forms and they are made available to bacteria only after autolysis of the tissue or after other enzymatic treatment. Thus folacin, N-10-formylfolic acid and citrovorum factor (N-5-formyltetrahydrofolic acid, folinic acid, leucovorin) have been established as products which arise in liver as the result of such treatments.

G. Toennies, E. Usdin, and P. M. Phillips (*J. Biol. Chem.* **221,** *855*, *865*, (*1956*)) have called attention to the character of the folacin-active materials in human blood. Interestingly enough, they have demonstrated that whole blood is almost devoid of free folacin as shown by microbiologic assay, but that significant amounts of the vitamin, as measured by a *Lactobacillus casei* growth assay, arise after the incubation of plasma and red blood cell hemolysates. Their results indicate that precursor materials in the cells can be converted to microbiologically active forms by an enzyme system (folacin conjugases) present in the plasma.

The precursor material from the red blood cells has been partially purified. The product, unstable in solution, can be protected by suitable concentrations of ascorbic acid. Ascorbic acid is likewise effective in preserving the labile citrovorum factor present in human urine (M. Silverman and R. C. Gardiner, *J. Bact.* **71,** *433* (*1956*)) and the protection afforded by this reducing agent suggests that the precursor in blood is a reduced derivative of folacin in which the N-5 position is unsubstituted.

Toennies and co-workers have attempted to establish the nature of the microbiologically active products which arise after digestion with serum of the folacin precursor in the blood cells. By the application of paper chromatograpy to samples obtained after digestion of concentrates of the precursor from blood cells with serum, the following have been eliminated as components of the precursor: folacin, citrovorum

factor, pteroyltriglutamic acid, N-10-formylfolic acid, anhydro-citrovorum factor, pteroyl-alpha-glutamylglutamic acid, thymine and thymidine. Although the composition of both the precursor compound and its components remains unclear, the factors which do arise after enzymatic digestion are characterized by high activity for *L. casei* and low activity for *Streptococcus fecalis* and *Leuconostoc citrovorum*.

The nature of the native forms of folacin in animal tissues is still uncertain. In the past, most of the contributions in this area have dealt with the isolation and characterization of various forms of the vitamin as they were released from precursors. The major problems now confronting investigators are the isolation of the intact (undegraded) precursors and the determination of their composition and structure.

RELATION OF LOW IODINE DIET TO THYROID TUMORS

Benign thyroid adenomas and nodular hyperplasia have been produced in the rat by prolonged feeding with goitrogens of the thiourea group of chemicals (G. L. Laqueur, *Cancer Research* **9,** *401* (*1949*); W. L. Money and R. W. Rawson, *Cancer* **3,** *110* (*1947*); K. E. Pashkis, A. Cantarow, and J. Stasney, *Cancer Research* **8,** *257* (*1948*)) and by the administration of radioactive iodine with partial destruction of the gland. It has been postulated in these cases that excessive release of thyrotropic hormone, due to suppression of thyroid hormone production, initiates tumor formation through chronic overstimulation of its target organ. Such a state of overstimulation of the thyroid can be produced without administering antithyroid drugs or damaging the gland with radioactive iodine, simply by feeding the experimental animals a diet low in iodine.

A. A. Axelrad and C. P. Leblond have reported several studies on the influence of iodine deficiency on the production of goiter, and on the incidence of thyroid tumors produced by this deficiency alone or with the administration of 2-acetylaminofluorene (*Canad. Med. Assn. J.* **67,** (*1952*); *Proc. Am. Assn. Cancer Research* **1,** *2* (*1953*)). In one of these studies of the induction of thyroid tumors in rats by a low iodine diet Axelrad and Leblond (*Cancer* **8,** *339* (*1955*)) used 98 Sherman albino rats which were fed a modification of Remington diet No. 342 providing approximately 0.7 micrograms of iodine per rat.

The animals were studied in three experiments. In each of these there were two groups, one fed the low iodine diet only and one to which the carcinogen 2-acetylaminofluorene was added in the following amounts: (1) 0.03 per cent from the fourth month on for thirty-nine days; (2) 0.03 per cent from the beginning of the experiment for thirty-seven days; (3) 0.04 per cent from the beginning of the experiment for 268 days. A separate group of rats was fed a diet adequate in iodine and was used as a control. Uniform conditions of light and temperature were used throughout the study. Five hours before being killed each animal received a dose of 10 to 20 micrograms of radioiodine subcutaneously.

The thyroid was removed with the trachea attached in order to avoid manipulating it, and the whole piece was fixed immediately. Groups of sections were then taken at several different levels and were studied with different staining techincs. Radioautographs were done as a qualitative measure of radioiodine accumulation in the tissue. The observations of microscopic examination were classified on the basis of cell morphology, staining characteristics, radioiodine contents, polarity, architectural grouping and invasiveness of tumor tissue. The criteria for neoplastic change in the tissues examined were pathologic change which is focal, cyto-

logically and architecturally distinct from the rest of the gland, and showing evidence of progressive growth.

When the results were analyzed it was found that thyroid nodules occurred in all the animals fed the low iodine diet for a sufficient length of time, regardless of whether 2-acetylaminofluorene was administered. The cells of all pathologic types described showed ability to invade surrounding tissue. Only one case of pulmonary metastasis occurred. It was noted that the incidence and time of occurrence of these nodules were not affected by a 0.03 per cent concentration of 2-acetylaminofluorene in the diet for a period of about five to six weeks in experiments 1 and 2. In experiment 3, however, in which the carcinogen was administered at a 0.04 per cent concentration for 268 days, a large number of tumors occurred in sites other than the thyroid and these animals had to be killed before the end of the experiment. The incidence of thyroid nodule formation in the animals in this group receiving the carcinogen remained the same as that in those receiving only the low iodine diet, leading to the conclusion that 2 - acetylaminofluorene merely accelerated the formation of the neoplastic lesions which would have formed on the low iodine diet alone. Only one of the rats fed a normal diet developed a neoplastic lesion in the thyroid.

In addition to studying the thyroid, the authors examined the pituitary glands of the animals used in this investigation. They found that in the animals fed the low iodine diet for nine and one-half months or more, there was frequently a significant increase in weight and gross hyperemia of this gland as compared to that of rats fed a normal diet. Gross tumors were seen in several instances. Systematic study was not made of these tumors, and the authors do not compare the carcinogen-fed animals with the others in relation to the occurrence of tumors in the pituitary.

Previous studies by Remington *et al.* and by Axelrad and Leblond (*loc. cit.*) failed to reveal any factor other than the prolonged iodine deficiency as a cause for the development of the thyroid nodules.

This work comes as an experimental confirmation of the observation that the introduction of the use of iodized salt in areas of endemic goiter has produced a drop in the incidence of death from cancer of the thyroid. As to the mechanism by which the thyroid lesions are produced in the experimental animals, it has been ascribed by several authors to the overproduction of thyrotropic hormone occurring secondarily to the low levels of thyroid hormone caused by the iodine-deficient diet.

No conclusions regarding the occurrence of tumors in the pituitary gland have been drawn and no attempt has been made by the authors to study the thyroid of rats receiving large amounts of thyrotropic hormone and maintained on a normal diet.

PELLAGRA AND THE COOKING OF CORN

Experimental work with rats has given some support to a hypothesis that the low incidence of pellagra in parts of Central America where there is a high intake of corn is related to the alkaline pretreatment of the corn with lime (*Nutrition Reviews* **10,** *105* (*1952*); **11,** *174* (*1953*)).

A study has now been carried out by G. A. Goldsmith, J. Gibbens, W. G. Unglaub and O. N. Miller (*Am. J. Clin. Nutrition* **4,** *151* (*1956*)) to investigate the nutritional status of human subjects kept under controlled conditions on a diet low in niacin and tryptophan, and containing a proportion of corn products prepared either from control or lime-cooked corn.

The control material was prepared from unenriched degerminated corn by milling

according to current North American practice. The lime-treated material, or "masa," was prepared in Guatemala by a traditional procedure: whole corn was simmered in water containing approximately 0.4 per cent lime for forty-five to sixty minutes, and left to stand for a further fourteen hours. The liquor was then poured off, and the residual material ground to a dough containing 60 per cent of moisture.

The two types of corn were incorporated into a diet already found by G. A. Goldsmith, H. L. Rosenthal, J. Gibbens, and W. G. Unglaub (*J. Nutrition* **56,** *371 (1955)*) to produce symptoms of niacin deficiency. This included 40 g. of dry matter in the breakfast menu coming from either corn grits or "masa" mush, and a further 40 g. coming in the form of bread with supper. The diets were supplemented each day with thiamine, riboflavin, pantothenic acid, pyridoxine and vitamin B_{12}.

Two adult women, who were maintained in a metabolism ward throughout the study but not confined to bed, were given a good varied diet for three weeks and then allocated one to each experimental diet for 140 days. The mean intakes over the experimental period for each individual were close to 1800 Calories, with approximately 190 mg. of tryptophan, and 5.3 mg. of niacin. The procedure for the assay of niacin in the diets was that proposed by W. A. Krehl, F. M. Strong and C. A. Elvehjem (*Ind. Eng. Chem. (Anal. Ed.)* **15,** *471 (1943)*) and included a preliminary autoclaving in 1 normal acid; this gave values of approximately 17.4 and 16 micrograms of niacin per gram of dry matter in the untreated corn and "masa" respectively, so that the intake of total niacin was similar regardless of the type of corn included in the diet.

The total daily urinary excretion of N-methylniacinamide and pyridone fell from initial values of over 4 mg. in each case to less than 2 mg. within thirty days of the beginning of the experimental diets. There was no consistent difference between the excretion of the subjects on the two diets, and no further downward trend in the next 100 days. After sixty days on the diets, signs suggestive of niacin deficiency appeared in each subject. These included slight atrophy of the tongue papillae, transient stomatitis and heartburn, but they failed to progress further in the remaining ninety days. The failure to develop typical pellagra may have been related to an increase in the niacin and tryptophan content of some canned components of the diets in the latter period.

Two other subjects were given the regimen including lime-treated corn. Their urinary excretion of niacin derivatives followed the same pattern, but in each case severe lesions characteristic of niacin deficiency appeared after thirty and forty-two days respectively. After two months on the diet symptoms of pellagra, that included glossitis, stomatitis and abdominal disorder, were sufficient to necessitate niacin therapy.

After two to three months of rehabilitation the same 2 subjects were then given a similar experimental diet containing whole Guatemalan corn that had been milled without previous lime-treatment, and in each case clinical signs appeared in a similar sequence to those obtained with the "masa" diet, with a similar fall in the excretion of niacin derivatives.

These studies failed, therefore, to demonstrate any mitigation in the pellagragenic properties of "masa" prepared by lime-treatment of corn, as compared with corn prepared by normal North American procedures. The authors point out, however, that corn provided only 15 to 20 per cent of the calories in the experimental regimens, whereas it may provide as much as 80 per cent of the dietary in parts of Central America where pellagra is rare. A difference in the balance of amino acids between the two types of diet might have a bearing on the problem.

There is also a suggestion from the present study and a comparison with the results of earlier work by the same group (Goldsmith *et al.* (*1955*), *loc. cit.*), that whole Guatemalan corn may be more active in producing niacin deficiency than degerminated United States corn. However, the existence of uncontrolled variables in the comparison leaves the question open to further investigation, and if later studies confirm the observation, there remains the further question as to whether the difference lies in the composition of the corn as first harvested, or in the practice of degermination before milling.

In another paper on the properties of lime-cooked corn, P. L. Pellett and B. S. Platt (*Nature* **177,** *422* (*1956*)) report that they have confirmed the findings of other workers (as discussed above) that replacement of raw corn by this material can prevent niacin deficiency in rats. They go on to point out, however, that in their laboratory the process of digesting corn in lime and discarding the liquor led to a serious loss of other members of the vitamin B-complex. They suggest that this could be an important limitation to the possible benefits derived from introducing such a cooking method in areas where corn, consumed in large quantities, may also be a significant source of these other vitamins.

Corn was treated with lime as described by R. O. Cravioto and co-workers (*Science* **102,** *9* (*1945*)) for the first stage in the preparation of Mexican tortillas. The procedure resulted in a fall in riboflavin content (measured on a 90 per cent dry matter basis) from 1.5 to 0.7 micrograms per gram, as measured by a microbiologic procedure, and a fall in thiamine content from 5.2 to 1.1 micrograms per gram, as determined by the thiochrome method. Feeding experiments with rats confirmed the fact of the depletion of these two vitamins. The result is, however, at variance with that of the original workers (Cravioto *et al.*, *loc. cit*), who found little loss of these two vitamins in their analyses of lime-treated products as compared with the original corn.

RIBONUCLEIC ACID AND PROTEIN SYNTHESIS

The science of nutrition has as a major objective the establishment of ideal conditions for protein synthesis. The practical result may be optimum growth rate in the young, the maintenance of nitrogen equilibrium in the adult, normal fetal development, or the synthesis of antibodies to fight bacterial infections, to cite a few examples. In order to ascertain the ideal cellular environmental conditions for protein synthesis, fundamental information concerning the mechanism of this synthesis is needed. Various experimental procedures have been employed. The classic nutritional approach has been to study the influence of diet on growth and nitrogen balance.

Since protein synthesis is in reality the incorporation of amino acids into the complex protein molecule, the advent of radioisotopes and heavy isotopes has offered a new experimental approach to its study. When an amino acid containing radioactive carbon (C^{14}) is incubated with tissue slices or homogenates it is possible to demonstrate the incorporation of the labeled amino acid into protein (*Nutrition Reviews* **6,** *198* (*1948*); **8,** *273* (*1950*)). Also, it is possible to inject the labeled amino acid into the intact animal and then measure its incorporation into tissue proteins.

By the use of these various technics considerable information concerning protein synthesis has accumulated. For example, it has been shown that the incorporation of amino acids into protein requires an energy source. This observation is in agreement

with the well known caloric requirement for protein synthesis in the animal. Of particular interest is the relationship of ribonucleic acid (RNA) to protein synthesis. Several lines of evidence indicate that RNA is an important regulator of protein synthesis. The concentration of RNA is greatest in cells which actively synthesize protein. E. F. Gale and J. P. Folkes (*Nature* **175**, *592* (*1955*)) offered additional and more direct evidence for this relationship. They found that the incorporation of labeled amino acids into protein by disrupted staphylococci was enhanced by RNA or its hydrolytic products.

Using a slightly different approach, J. W. Littlefield, E. B. Keller, J. Gross, and P. C. Zamecnik (*J. Biol. Chem.* **217**, *111* (*1955*)) present interesting data relating RNA to protein synthesis. It is well known that when an animal is injected with a radioactive amino acid the proteins of different regions of the cell are labeled at different rates. The protein most active in this regard is that present in the microsomes. The microsomes are small particles which may be separated from the cellular cytoplasm by differential centrifugation. Littlefield *et al.* (*loc. cit.*) injected rats with C^{14}-labeled amino acids and excised the livers at varying time intervals thereafter. The livers were then homogenized in isotonic sucrose solution and the microsomes were separated by centrifugation. They noted, as expected, that the injected amino acids were rapidly incorporated into the protein of the microsomes. The microsomes were then further fractionated by the use of sodium desoxycholate. This reagent precipitated a fraction which was quite high in RNA. The RNA content of this desoxycholate-insoluble fraction was approximately 50 per cent and the fraction was considered to be ribonucleoprotein. The desoxycholate-soluble fraction was mostly protein and contained only small quantities of RNA. Injected amino acids were incorporated much more rapidly into the protein of the ribonucleoprotein than into the remaining protein of the microsomes. The protein of the cell exclusive of the microsomes was labeled at a still lower rate.

These results suggest that the first step in protein synthesis may be the incorporation of amino acids into the protein of ribonucleoprotein. Fragments of this protein may then be transferred to other protein. Although these considerations are speculative the data certainly re-emphasize the important relationship of RNA to protein synthesis.

In a more recent report E. B. Keller and P. C. Zamecnik (*J. Biol. Chem.* **221**, *45* (*1956*)) describe *in vitro* experiments concerning the incorporation of amino acids into protein. When C^{14}-labeled amino acids were incubated with microsomes and the soluble cell fraction, the amino acids were incorporated into protein. The soluble cell fraction is that portion of the cytoplasm which does not sediment when subjected to centrifugation under the conditions used to sediment microsomes. This system required adenosine triphosphate (ATP) and an ATP-generating system such as creatine phosphate. When the soluble cell fraction was further purified by precipitation of the active proteins by pH adjustment it was no longer active under the conditions employed in the crude system. However, when guanosine diphosphate (GDP) or guanosine triphosphate (GTP) was added, amino acids were incorporated into protein. GDP and GTP are nucleotides similar to those present in RNA.

These results permit a possible formulation of several of the steps in protein synthesis. The first step appears to be carboxyl activation of the amino acid and requires ATP and enzymes from the soluble fraction of the cell (F. Lipmann, *Advances in Enzymol.* **1**, *99* (*1941*)). The activated amino acid in the presence of GDP or GTP may then be incorporated into the protein of the ribonucleoprotein of the microsomes. From

here polypeptide chains may be transferred to participate in the synthesis of other cellular protein. One of the most important problems in protein synthesis is how the cell synthesizes the many specific proteins (enzymes) with definite orders of their amino acids, and here ribonucleic acid itself may be the template.

AORTIC RUPTURE FROM FEEDING SWEET PEAS

Previous reports of dissecting aneurysms, characterized by splitting of the media of the aorta, in rats fed sweet pea meal (*Lathyrus ordoratus*) have stressed the necessity of limiting the dietary protein intake (T. E. Bachhuber and J. J. Lalich, *Arch. Path.* **59,** *247* (*1955*); W. Dasler, *Proc. Soc. Exp. Biol. Med.* **85,** *485* (*1954*)). Further abnormalities produced by sweet pea feeding have included skeletal deformity, sometimes leading to paralysis, and hernia formation (B. J. Geiger, H. Steenbock and H. T. Parsons, *J. Nutrition* **6,** *427* (*1933*); *Nutrition Reviews* **14,** *65*, *303* (*1956*)). These earlier studies have left unanswered many questions regarding the cellular and biochemical mechanisms by which this vascular disease is induced. Two recent studies advance our knowledge of the factors responsible for this type of disintegration of the largest blood vessel in the body.

D. G. Walker and Z. T. Wiitschafter (*Arch. Path.* **61,** *125* (*1956*)) have studied the histologic development of these lesions under conditions which resulted in high incidence of lesions. Using a whole wheat, casein and milk powder control ration which was mixed half and half with crude ground sweet pea seeds, they were able to produce severe vascular disease in the majority of their animals. Eight of 24 rats died of aortic rupture and apparently most of the remaining 16 animals showed definite lesions. In contrast to previous reports, these investigators describe severe lesions throughout the aorta rather than in the arch only (T. E. Bachhuber and J. J. Lalich, *Ibid.* **59,** *247* (*1955*)). They also found evidences of elastic tissue destruction and accompanying fibroblast proliferation in other parts of the vascular system, including the pulmonary arteries, the coronary arteries and the atrial endocardium. These are different from the results reported by I. V. Ponseti and W. A. Baird (*Am. J. Path.* **28,** *1059*, (*1952*); *Nutrition Reviews* **14,** *223* (*1956*)).

J. J. Lalich (*Arch. Path.* **61,** *520* (*1956*)) has contributed additional knowledge to the nutritional understanding of odoratism. Utilizing the recent observation that beta-aminopropionitrile (BAPN) may be responsible for some of the toxicity of *Lathyrus odoratus* meal (E. D. Schilling and F. M. Strong, *J. Am. Chem. Soc.* **76,** *2848* (*1954*); *Nutrition Reviews, loc. cit.*)), Lalich has carried out a series of assays to see if other nutritional factors will protect the animal or increase the damage from BAPN. Giving BAPN in a dose of 1.9 g. per Kilogram per day in a 12 per cent casein diet, he tested various amino acid and vitamin supplements for their effects on weight gain, skeletal deformity, hernia formation and aortic rupture during a seven-week period. Skeletal deformity developed in all these animals and weight gain was poor as compared to the controls, although aortic rupture was infrequent, occurring in only 4 of the 51 rats.

In a second experiment Lalich utilized a higher dose of a more stable salt of BAPN and was able to induce aortic rupture in 19 of 36 rats. Hernia formation was rare in the rats of the second experiment as compared to its occurrence in the first.

In general, the results reported by Lalich show that weight gain is best in those BAPN-treated animals receiving supplements of tryptophan, glutamic acid, or

methionine along with excess B-vitamins. Hernia formation appeared to occur more frequently in rats receiving additional glycine, alanine, or glutamic acid. None of the additional dietary ingredients appeared to protect against skeletal deformity or aortic rupture. In fact, aortic rupture seemed to be more frequent when methionine was present as a supplement, although control groups of animals receiving only BAPN in addition to the basal diet were not included in the study.

Together, these two recent investigations indicate that the toxic component of the sweet pea meal is beta-aminopropionitrile. It appears to be capable of producing all of the pathologic effects of the crude meal, provided that it is given in a stable form. The toxic mechanism is not yet well defined but it seems probable that lysis, or the disintegration of elastic tissue throughout the body, is an important characteristic of this toxicity. Elastic tissue is an important and irreplaceable component of the connective tissue. Its degeneration may be an important factor in the aging of many tissues of the body, notably the lungs, the heart valves, the blood vessels, the bones and the joints. A search should be made for other nutritional or metabolic substances which resemble the nitriles and which may be contributing to elastic tissue degeneration in man.

DIET SELECTION DURING STRESS

The eating behavior of animals given a choice of diets varying in nutritional content has been discussed many times (*Nutrition Reviews* **2,** *199* (*1944*); **3,** *341* (*1945*); **5,** *24* (*1947*); **7,** *175*, *229* (*1949*); **9,** *28* (*1951*); **10,** *303* (*1952*); **11,** *88* (*1953*); **12,** *117* (*1954*)). The aim of most investigations has been to determine whether selections made by the animals may be correlated with their physiologic needs. However, the concensus seems to be that numerous factors other than nutritional requirements have entered into the experiments, and animals will often sample a variety of diet components apparently regardless of flavor. Some evidence has been presented which indicates a preference for certain general food stuffs (such as fats in the case of hereditarily obese mice), but discrimination between various purified salts, vitamins, or other nutrients has not been satisfactorily demonstrated.

In studies of the Norway rat, C. P. Richter *et al.* (*Am. J. Physiol.* **122,** *734* (*1938*)) have shown that this animal will grow and maintain itself on a self-selected diet of purified foodstuffs, possibly for nonspecific reasons. However, W. J. Griffiths (*Science* **112,** *786* (*1950*)) has demonstrated that the incidence of susceptibility to sound-induced seizures is significantly reduced in some of these rats by allowing them to subsist on a self-selected diet. In other studies Griffiths and coworkers (*Ibid.* **118,** *780* (*1953*); *Japan Psychol. Research* **1,** *9* (*1954*)) found that rats subjected to the stress of forced swimming chose dextrose over saccharin, and that such stresses as electric shocks and fighting induced diet choices different from the "resting" selections. In a further exploration of the relation of stress to food selection, Griffiths has investigated the nutrient selections of domestic Norway rats subjected to the stress of treadmill running (*Ann. N. Y. Acad. Sciences* **67,** *1* (*1956*)).

Twelve adult male rats were used as subjects, 2 of which died of convulsive seizures, apparently because of failure to adapt to the treadmill situation. The self-selected diet utilized in the study consisted of the following substances, contained separately in graduated 100 ml. inverted drinking tubes: 1 per cent potassium chloride, 2 per cent calcium lactate, 3 per cent sodium chloride, 4 per cent sodium phosphate, 1.6 per cent

magnesium chloride, 0.02 per cent vitamin B_6, 0.02 per cent thiamine, 0.01 per cent calcium pantothenate, 0.1 per cent niacinamide, 0.5 per cent choline chloride, 0.00125 per cent riboflavin, distilled water, olive oil and cod liver oil. Dextrose and casein were provided as solids. For a period of twenty-two days the animals were allowed to adjust to self-selection of foodstuffs. They were then exposed to the treadmill on alternate days for twelve days. Diets were also recorded during a ten-day rest period following the treadmill-running period.

Sodium chloride, potassium chloride, sodium phosphate, calcium pantothenate and distilled water were selected in increased amounts during the twelve days of treadmill-running as compared to selections made during the last eight days of the adaption period. All differences were found to be statistically valid at the 5 per cent level of significance.

When selections made on days when the animals were not put on the treadmill were compared with those made during actual treadmill-running it was found that sodium chloride, sodium phosphate, and calcium pantothenate were taken in significantly greater amounts on treadmill-running days, while calcium lactate and distilled water were taken in significantly smaller amounts.

Sodium chloride, potassium chloride, sodium phosphate and calcium pantothenate were selected in smaller amounts during the rest period as compared to the treadmill-running period.

The author believes that the results of this study support the hypothesis that self-selection of diet offers a useful means of understanding the nature of processes underlying behavior. This implies that the diet selections were predictable acts induced by specific physiologic needs. The author particularly stresses the selection of calcium pantothenate, pointing out that this vitamin is concerned with the metabolism of hormones of the adrenal cortex.

It is possible that the ability of rats to make certain grossly appropriate selections of food and water will determine the prognosis of survival in a natural environment. Even in man, certain situations stimulate the intake of more or less than the usual amount of water and encourage or discourage the intake of general categories of foodstuffs. However, one cannot but question the biologic significance of some of the particular choices made in this experiment. Although saline solutions were selected preferentially during the treadmill-running period, all of these, with the exception of calcium pantothenate, were selected to about the same degree as distilled water. In order to satisfy increased thirst following treadmill-running, the animals may have merely sampled the least distasteful of various solutions offered. Thus, it would be of interest to determine if the dietary selections could be altered by substitution of tap water for the "flat"-tasting distilled water.

A possible alternative explanation for the seemingly increased choice of calcium pantothenate solution during treadmill-running resides in its low concentration, which might have been just sufficient to make the distilled water (the solvent) more palatable. Furthermore, even if the animals are able to distinguish between potassium chloride, sodium chloride and sodium phosphate on the one hand and pure calcium pantothenate on the other, this ability would seem to be an unpractical one since the rat obviously does not encounter pure calcium pantothenate in nature. It is also far from clear whether the food choices were based on physiologic stimuli arising from stress, exercise, loss of body water, or still other causes.

Apparently, the final conclusions cannot as yet be reached whether the animal can truly discriminate between pure nutrients normally present in low concentration in the diet, whether the discrimination is actually a result of the experimentally-induced physiologic stimuli, and whether the discrimination is a useful response to the stimulus.

ISOMERISM OF VITAMIN A

Although the relationship of vitamin A to its several precursors has been known for some time, the multiple nature of the vitamin itself was not fully appreciated until the modern technics of chromatography and spectrophotometric analysis permitted the separation and characterization of these closely related compounds. Thus, although the synthetic vitamin A possessing all 5 of its double bonds in the trans configuration was also shown to be the major naturally occurring form, it soon became evident that the all-trans isomer was not the only vitamin A present in many natural sources. For example, neovitamin A, an isomer found in various fish liver oils and with about 85 per cent of the potency of the all-trans for rat growth, was ascertained to be the 13-cis (cis double bond nearest the hydroxyl group) (*Nutrition Reviews* **9,** *315 (1951)*). It has been suspected that other cis isomers occur naturally since many concentrates show greater amounts of vitamin by the Carr-Price reaction (which does not readily distinguish the isomers) than by biologic tests in which the all-trans isomer is the most active. The possible cis isomers are the 7-, 9-, 11- and 13-cis and the 9:13- and 11:13 di-cis vitamins A. Methods of estimation of these isomers have, however, been difficult, specialized and of limited application.

In an investigation of the isomerism of vitamin A in marine crustacea, P. A. Plack (*Biochem. J.* **64,** *56 (1956)*) utilized the familiar reaction of maleic anhydride with dienes not only to estimate the proportion of certain isomers but also as an aid in isolation. It was found that some of the isomers (trans configuration at double bonds 11 and 13) react rapidly and completely with the reagent while the others (such as the 13 cis) may react at less than one per cent of this rate. Thus, the rate of disappearance of vitamin A (as measured by the Carr-Price reaction) by reaction with maleic anhydride could be used to determine the proportion of "fast"- and "slow"-reacting components. Moreover, after a short reaction period, the unreacted "slow"-reacting isomers could be extracted from the water-soluble adducts of maleic anhydride and the "fast"-reacting isomers, thus enabling a fairly complete separation to be made.

Using these methods, Plack found that the vitamin A of several marine crustacea, such as *Meganyctiphanes norvegica*, was composed of 70 to 95 per cent of "slow"-reacting components, while that of whale-liver oil contained about 30 per cent of these isomers.

Some further differentiation of the components of the crustacean vitamin A was effected by noting the shifts in wavelength and extinction coefficient of their absorption maximum in the ultra violet by isomerization with iodine (P. A. Plack, L. R. Fisher, K. M. Henry, and S. K. Kon, *Biochem. J.* **64,** *17 p. (1956)*). This shift of wave length from 315 to 325 millimicrons to 325 millimicrons and the one and one-half-fold increase in extinction coefficient are consistent with previous findings (R. Hubbard and G. Wald, *J. Gen. Physiol.* **36,** *269 (1952–3)*) that the bulk of the vitamin A from this source is the neovitamin Ab or the 11-cis.

In a similar study of the vitamin A of fresh-water fish, further complication would, of course, be introduced by the presence of vitamin A_2 (which contains one more double bond than vitamin A_1), and its possible configurational isomers. As a matter of fact, this compound is also found in marine fish liver oils (*see, for example*, H. R. Cama, F. D. Collins, and R. A. Morton, *Biochem. J.* **50,** *48 (1951)*).

Recently, a study of the proportion of vitamins A_1 and A_2 in fresh-water fish-liver oils from Indian waters has been carried out by S. Balasundaram, H. R. Cama, R. R. Sundaresan, and T. N. R. Varma (*Biochem. J.* **64,** *150 (1956)*), using the reaction with antimony trichloride (Carr-Price) which

gives an absorption maximum at 620 millimicrons with vitamin A_1 and at 693 millimicrons with vitamin A_2. Chromatographic separation (on alumina) was also used to determine the proportions of ester and alcohol in the various oils.

The oils containing a high proportion of vitamin A_2 and little vitamin A_1 (about a 10:1 ratio) were found to be from predatory fish, whereas oils from fish of vegetarian habits were comparatively low in vitamin A_2 (about a 1:2 ratio). It was also shown that vitamin A_2 was present largely as the ester while vitamin A_1 occurred entirely as the alcohol.

Although these investigations have served to focus attention on the complexity of the vitamin A family and to elucidate, in some cases, the composition of some of the natural mixtures, they have left many questions unanswered. More precise methods are needed to determine exactly which configurational isomers of vitamin A_1 may be present in natural oils. The configurational isomerism of vitamin A_2 has not been investigated, and the biogenesis of this compound is by no means clear.

Studies such as these not only contribute greatly to our knowledge of the structural details of the vitamin A family but also aid in our understanding of the functions of this vitamin and its role in nutrition.

THYROXINE AND OXIDATIVE PHOSPHORYLATION

The well-known role of the thyroid hormone in regulating the basal metabolic rate has not been explained in precise biochemical terms. Recently, considerable attention has been devoted to the influence of thyroxine and other thyroid-active substances on oxidative phosphorylation. The energy derived from oxidative reactions in the body is utilized, for the most part, in the formation of high energy phosphate bonds. In the presence of such energy-yielding reactions, adenosine diphosphate (ADP) is converted to adenosine triphosphate (ATP). The high energy phosphate bond so formed may in turn be used in muscle contraction, protein synthesis, and other energy-requiring processes.

The rate with which the oxidative reactions proceed is directly proportional to the quantity of ADP available. If the formation of ATP from ADP is blocked, the energy from oxidative reactions cannot be utilized efficiently, and since the level of ADP will remain high the oxidative reactions continue to proceed at an elevated rate. Such a situation is referred to as the uncoupling of oxidative phosphorylation. The ratio of high energy phosphate bonds formed to oxygen consumed in oxidation reactions is referred to as the P/O ratio. Thus, a reduced P/O ratio indicates an uncoupling of oxidative phosphorylation and a consequent reduction in the efficiency of utilization of energy. The excess energy in such a circumstance would be dissipated mostly as heat.

Many substances chemically related to thyroxine are known to uncouple oxidative phosphorylation, one of the best examples being dinitrophenol. Also one of the most striking of the physiological effects of excess thyroid hormone, an increased heat production, could be explained nicely on the basis of an uncoupled oxidative phosphorylation. G. F. Maley and H. A. Lardy (*J. Biol. Chem.* **204**, *435 (1953)*) obtained evidence that thyroxine and triiodothyrionine uncoupled oxidation phosphorylation. The *in vitro* addition of either of these hormones to rat kidney mitochondria oxidizing glutamate led to a reduction in P/O ratios. Carefully defined experimental conditions were required to produce the effect, and the quantity of thyroxine required was considerably in excess of normal tissue levels.

F. L. Hoch and F. Lipmann (*Proc. Nat.*

Acad. Sci. **40,** *909 (1954)*) studied the effects on P/O ratios of injecting thyroxine into animals. Liver mitochondria from thyroxine-injected animals exhibited a lowered P/O ratio while oxidizing a variety of substrates. Other evidence for an interference of thyroxine with oxidative phosphorylation was obtained. With normal liver mitochondria oxidizing a substrate, the addition of a phosphate acceptor, such as glucose and hexokinase, stimulates respiration. ATP and the enzyme hexokinase phosphorylate glucose to glucose-1-phosphate and produce ADP. This ADP, in turn, stimulates respiration. It was found that this so-called acceptor effect was absent, or greatly reduced, in livers from thyroxine injected animals. It seems likely that the original phosphorylation of ADP was reduced in the thyroxine-treated animals and thus, without any phosphate acceptor, the ADP levels remained high.

Such results as these have led to the suggestion that one important biochemical effect of the thyroid hormone is to uncouple oxidative phosphorylation. The recent reports of D. F. Tapley (*J. Biol. Chem.* **222,** *325 (1956)*) and D. F. Tapley and C. Cooper (*J. Biol. Chem.* **222,** *341 (1956)*) indicate that this effect of thyroxine or triiodothyrionine may be indirect, and may be the result of changes in permeability or structure of the mitochondrial membrane. In the first experiments, a study was made of the effects of various agents on the swelling of isolated rat liver mitochondria. The experimental conditions were such that no oxidation or phosphorylation were occurring. The swelling of the mitochondria was due to the passage of water into the particle, and could be followed conveniently by optical density measurements. A diverse group of substances influenced the degree of swelling, but of particular interest was the effect of thyroxine. Concentrations of thyroxine as low as 10^{-6} M led to a prompt increase in the degree of swelling. This is a considerably lower concentration of thyroxine than is required to lower P/O ratios significantly.

In other experiments mitochondria were prepared from livers of normal, hyperthyroid, and hypothyroid rats and the degree of swelling was measured. It was found that the mitochondria from hyperthyroid rats swelled at a greater than normal rate, while those from hypothyroid rats swelled at a considerably reduced rate. From these experiments it appears that the thyroid hormone may exert a significant effect on the integrity of the mitochondrial membrane.

It seemed possible that the earlier observed effects of thyroxine on P/O ratios might be the result of changes in permeability of the mitochondrial membrane. To test this hypothesis, rat liver mitochondria were fractionated in such a way that the membrane was disrupted and an active enzyme fraction was obtained. This fraction, which was considered to be a sub-unit of the mitochondria, would actively oxidize β-hydroxybutyrate and form high energy phosphate bonds. The addition of thyroxine to this preparation did not affect P/O ratios, even when added in concentrations considerably greater than were effective in intact mitochondrial preparations. Triiodothyrionine was likewise ineffective in uncoupling oxidative phosphorylation in the mitochondrial extract. In contrast, the uncoupling agent dinitrophenol was as effective in the extract as in intact mitochondria.

These results show that, whereas thyroxine or triiodothyrionine uncouple oxidative phosphorylation in intact mitochondria, no such effect is observed when the mitochondrial membrane has been disrupted. This observation, taken in conjunction with the observed effect of thyroxine on swelling of the mitochondria, strongly suggests that the effect of thyroxine on oxidative phosphorylation is indirect and related to changes in the structure of the mitochondrial membrane.

NOTES

Nutrient Composition of Frozen Foods

M. Burger and co-workers (*J. Agr. Food Chem.* **4**, *418* (*1956*)) have reported the results of an extensive survey on the vitamin, mineral and proximate composition of frozen foods. During the 1953 and 1954 production seasons, 796 sets of samples representing 14 frozen fruits, 7 frozen juices and 30 frozen vegetables were collected at 150 freezing plants in various production areas in the United States and Hawaii. Representative sampling was undertaken in order to have a fair weighting for such variables as weather, crop varieties, harvesting, processing, packaging and grades of the products. Evaluations of proximate components were made by acceptable procedures for total solids, ash, ether extract, protein, crude fiber, carbohydrate (by difference) and calories. Calcium, total iron, phosphorus, magnesium, potassium and sodium determinations were made. The following vitamin concentrations were determined: reduced and dehydroascorbic acid, beta-carotene, folacin, niacin, pantothenic acid, riboflavin, thiamine and vitamin B_6. For all foods and each constituent, the values were presented in terms of the average and also the minimum and maximum values observed.

Undoubtedly this paper will be a standard reference for everyone concerned with the planning of diets in which frozen foods are used extensively and also for those who need to calculate food consumption data for experimental subjects or patients. The variation in composition with respect to nutrients is worthy of comment. For example, the range of total ascorbic acid in apricots varied from 6.1 to 100.3 mg. per 100 g., with the average at 27.5 mg. per 100 g., while the variation for sliced peaches was 11.2 to 76.1 mg. per 100 g., with an average of 39.8 mg. per 100 g. Burger and co-workers draw attention to the fact that ascorbic acid was used in processing both these products. The average total ascorbic acid contents of apricots and peaches packed without added ascorbic acid were 8.6 and 11.2 mg. per 100 g. respectively, while the averages for samples packed with added ascorbic acid were 100.3 and 76.1 mg. per 100 g., respectively. Larger variations in sodium content were also observed as a result of whether sodium chloride was used in the packing process of such foods as apples, lima beans, orange juice, succotash, black-eyed peas, etc. Other less readily predictable variations were observed: squash varied in iron content from 0.8 to 4.5 mg. per 100 g. and in vitamin B_6 content from 0.076 to 0.245 mg. per 100 g., while the iron content of cut corn varied from 0.2 to 1.9 mg. per 100 g. and its calcium content varied from 0.5 to 8.5 mg. per 100 g.

It is interesting to compare the average values for various nutrients in these frozen food samples with those values for fresh and canned foods tabulated by A. D. Bowes and C. F. Church ("Food Values of Portions Commonly Used," *8th edition, College Offset Press, Philadelphia* (*1956*)). For example, the average value for total ascorbic acid in 46 samples of frozen orange juice was 47.2 mg. per 100 g.; in comparison, the value given by Bowes and Church for the ascorbic acid content of a fresh orange is 49 mg. per 100 g. and for canned sweetened or unsweetened orange juice is 42 mg. per 100 g. The protein content of frozen baby lima beans is 7.6 per cent, while the value of Bowes and Church for raw green lima beans is 7.5 per cent. Many other comparisons could be made.

Perhaps it would be well to point out that the nutrient values of frozen foods at the freezing plant do not necessarily represent the nutrient intakes actually obtained by the consumer. These obviously depend on the methods of storage, handling and cooking. The same comments would of course apply to the procedures used for fresh and canned foods.

Recent Nutrition Books

Animal Nutrition. Leonard A. Maynard and John K. Loosli. 4th edition. McGraw-Hill Book Company, Inc., New York, 1956. Pp. 484.

Grouping of Raw Agricultural Commodities for Residue Comparisons and the Simplification of Applications for Tolerances. Report prepared by the Pesticides Subcommittee of the Food Protection Committee. Food and Nutrition Board, National Academy of Sciences—National Research Council, Washington 25, D. C., October 1955. Pp. 6.

Protein Malnutrition in Brazil. J. Waterlow and A. Vergara. Published by Food and Agriculture Organization of the United Nations, Rome, Italy, March 1956. Price: 50 cents.

The Soil Bank—Objectives; Administration in 1956. Office of Information, United States Department of Agriculture, Washington, D. C., June 1956.

Dietary Studies of Montana Fifteen-Year-Olds and of Montana College Freshmen. Bulletin 518. The Human Nutrition Research Branch, United States Department of Agriculture and Montana State College, Agricultural Experiment Station, Bozeman, Montana, April 1956.

Report of the Panel of Composition and Nutritive Value of Flour. Sir Henry Cohen, Chairman, A. C. Chibnall, J. H. Gaddum, R. A. Morton, and L. J. Witts. Presented to Parliament by the Secretary of State for Scotland, the Minister of Agriculture, Fisheries and Food and the Minister of Health by Command of Her Majesty, May 1956. Her Majesty's Stationery Office, London.

Polysaccharides in Biology. Transactions of the First Conference, April 27, 28 and 29, 1955. Contents: Problems of Communication: Nomenclature; Problems of Classification; Bacterial Polysaccharides; Blood Group Substances. Edited by George F. Springer. Sponsored by the Josiah Macy Jr. Foundation, New York, N. Y.

Vitamina E. Atti del terzo Congresso Internazionale, Venezia 1955. Comitato Organizzatore del III Congresso Internazionale della Vitamina E, Venezia, 5–8 September 1955: President, Carlo Foà; Vice Presidents, Emilio Trabucchi and Pietro Rondoni; Secretary, Emilio Raverdino.

Contents: A tribute by K. E. Mason to Sir Jack Drummond, under whose guidance the first International Congress on Vitamin E was organized; programs of preceding conferences, etc., and foreword by Professor Foà; vitamin E and metabolic processes; vitamin E and its relations to other vitamins, hormones and enzyme systems; vitamin E and cardiovascular system; vitamin E and nervous and muscular system; vitamin E in veterinary science. Impresso nel mese di aprile 1956, dalla stamperia Valdonega di Verona.

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Mechanisms of Congenital Malformation. Proceedings of the Second Scientific Conference of the Association for the Aid of Crippled Children. Printed by The John B. Watkins Company, 9 Murray Street, New York 7, N. Y. Pp. 137. Price: $3.00.

The Use of Chemical Additives in Food Processing. Prepared by the Food Protection Committee of the Food and Nutrition Board. National Academy of Sciences—National Research Council Publication 398, Washington 25, D. C., February 1956. Pp. 86. Price: $2.00.

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